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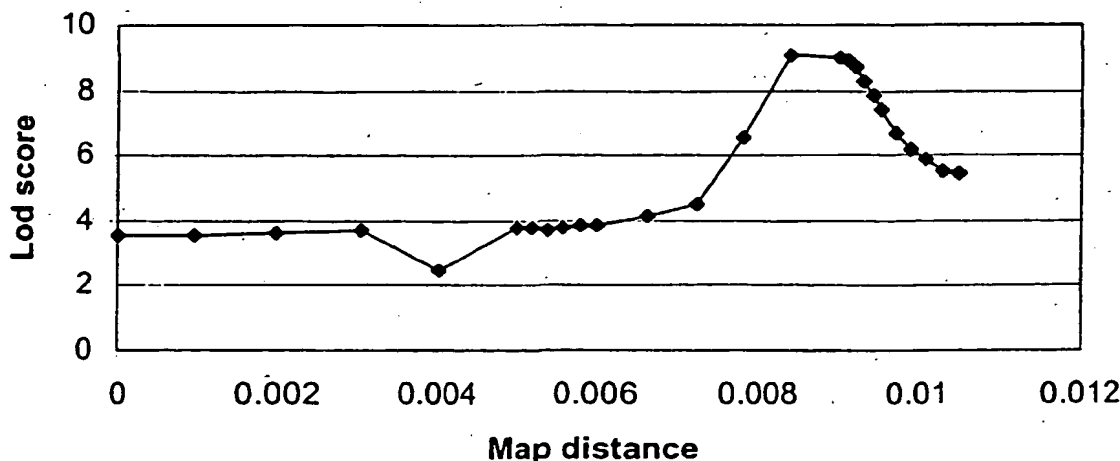
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(54) Title: VARIANTS OF THE GAMMA CHAIN OF AMPK, DNA SEQUENCES ENCODING THE SAME, AND USES THEREOF



(57) Abstract: The invention concerns variants of the gamma chain of vertebrate AMP-activated kinase (AMPK), as well as nucleic acid sequences encoding said variants and use thereof for the diagnosis or treatment of dysfunction of energy metabolisms.

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*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

VARIANTS OF THE GAMMA CHAIN OF AMPK, DNA SEQUENCES  
ENCODING THE SAME, AND USES THEREOF.

The present invention relates to new variants  
of the  $\gamma$  chain of AMP-activated protein kinase (AMPK), to  
5 genes encoding said variants and to uses thereof.

AMPK has a key role in regulating the energy  
metabolism in the eukaryotic cell (HARDIE et al., Annu.  
Rev. Biochem., 67, 821-855, 1998; KEMP et al., TIBS, 24,  
22-25, 1999). Mammalian AMPK is a heterotrimeric complex  
10 comprising a catalytic  $\alpha$  subunit and two non-catalytic  $\beta$   
and  $\gamma$  subunits that regulate the activity of the  $\alpha$   
subunit. The yeast homologue (denoted SNF1) of this  
enzyme complex is well characterised; it comprises a  
catalytic chain (Snf1) corresponding to the mammalian  $\alpha$   
15 subunit, and regulatory subunits: Sip1, Sip2 and Gal83  
correspond to the mammalian  $\beta$  subunit, and Snf4  
correspond to the mammalian  $\gamma$  subunit. Sequence data show  
that AMPK homologues exist also in *Caenorhabditis elegans*  
and *Drosophila*.

20 It has been observed that mutations in yeast  
SNF1 and SNF4 cause defects in the transcription of  
glucose-repressed genes, sporulation, thermotolerance,  
peroxisome biogenesis, and glycogen storage.

In the mammalian cells, AMPK has been proposed  
25 to act as a "fuel gauge". It is activated by an increase  
in the AMP:ATP ratio, resulting from cellular stresses  
such as heat shock and depletion of glucose and ATP.  
Activated AMPK turns on ATP-producing pathways (e.g.  
fatty acid oxidisation) and inhibits ATP-consuming  
30 pathways (e.g. fatty acid and cholesterol synthesis),  
through phosphorylation of the enzymes acetyl-CoA  
carboxylase and hydroxymethylglutaryl-CoA (HMG-CoA)  
reductase. It has also been reported to inactivate in  
vitro glycogen synthase, the key regulatory enzyme of  
35 glycogen synthesis, by phosphorylation (HARDIE et al.,

1998, *supra*); however, whether glycogen synthase is a physiological target of AMPK *in vivo* remained unclear.

Several isoforms of the three different AMPK subunits are present in mammals. In humans, *PRKAA1* on  
5 human chromosome (HSA) 5p12 and *PRKAA2* on HSA1p31  
respectively encode isoforms  $\alpha 1$  and  $\alpha 2$  of the  $\alpha$  subunit,  
*PRKAB1* on HSA12q24.1 and *PRKAB2* (not yet mapped)  
respectively encode isoforms  $\beta 1$  and  $\beta 2$  of the  $\beta$  subunit,  
and *PRKAG1* on HSA12q13.1 and *PRKAG2* on HSA7q35-q36  
10 respectively encode isoforms  $\gamma 1$  and  $\gamma 2$  of the  $\gamma$  subunit  
(OMIM database, <http://www.ncbi.nlm.nih.gov/omim/>, July  
1999). HARDIE et al., [1998, *supra*] also mention the  
existence of a third isoform ( $\gamma 3$ ) of the  $\gamma$  subunit of AMPK  
but do not provide any information about it. Analysis of  
15 the sequences of these  $\gamma$  subunits shows that they are  
essentially composed of four cystathione  $\beta$  synthase (CBS)  
domains whose function is unknown. No phenotypic effect  
resulting from a mutation in either of the AMPK subunits  
has yet been documented.

20 On the other hand, it has been observed that  
most Hampshire pigs have a high intramuscular glycogen  
concentration. In these pigs, glycogenolysis which occurs  
after slaughtering leads to an important decrease of the  
pH, resulting in acid meat having a reduced water-holding  
25 capacity and giving a reduced yield of cured cooked ham.

The locus (named *RN*) associated with high  
muscular content of glycogen was first identified by  
family segregation analysis of phenotypic data from  
Hampshire pigs (LE ROY et al., Genet. Res., 55, 33-40,  
30 1990). A fully dominant allele, *RN*, correlated with high  
glycogen content occurs at a high frequency in most  
Hampshire populations while pigs from other breeds are  
assumed to be homozygous for the normal, recessive *rn*<sup>+</sup>  
allele. Subsequent studies showed that *RN* carriers have a  
35 large increase (about 70%) of glycogen in skeletal muscle

but not in liver (MONIN et al., in 38<sup>th</sup> ICOMST, Clermont-Ferrand, FRANCE, 1992).

The large difference in glycogen content between RN and rn<sup>+</sup> pigs leads to marked differences in meat quality and technological yield (ENFÄLT et al., J. Anim. Sci., 75, 2924-2935, 1997). The RN allele is therefore of considerable economical significance in the pig industry and most breeding companies would like to reduce or eliminate this dominant mutation.

10 The RN phenotype can be determined by measuring the glycolytic potential in muscle biopsies from live animals, or after slaughter (MONIN et al., Meat Science, 13, 49-63, 1985). However, this method has severe limitations for application in practical breeding programs. The accuracy of the test is not 100%: as there is some overlap in the phenotypic distribution of RN and rn<sup>+</sup>, the test is not able to distinguish RN/RN homozygotes and RN/rn<sup>+</sup> heterozygotes. Further, the sampling of muscle biopsies on live animals is invasive and costly.

20 Thus, there is a strong need for the development of a simple diagnostic DNA test for the RN locus. Moreover, the dramatic phenotypic effect of the RN gene in pigs implies that this gene has an important role in the regulation of carbohydrate metabolism in skeletal muscle in other vertebrates, in particular mammals.

25 Skeletal muscle and liver are the two major reservoirs of glycogen in mammals and the observation of an increased muscular glycogen while liver glycogen is normal suggests that the RN phenotype maybe due to a mutation in a gene expressed in muscle but not in liver. The inventors have previously reported that the RN gene is located on pig chromosome 15 (MILAN et al., Mamm. Genome, 7, 47-51, 1996; MARIANI et al., Mamm. Genome, 7, 30 52-54, 1996; LOOFT et al., Genetics Selection Evolution, 35 28, 437-442, 1996). They have now discovered that the RN

allele is associated with a non-conservative mutation in a gene encoding a new muscle-specific isoform of the AMP-activated protein kinase (AMPK)  $\gamma$  chain.

5 The various aspects of the present invention are based upon the discovery and characterisation of this mutation and the identification and isolation of the mutant gene.

According to the invention it is shown that a mutation in a  $\gamma$  chain of AMPK results in an altered regulation of carbohydrate metabolism, demonstrating that AMPK is an essential component of said metabolism. It is also provided a nucleic acid sequence encoding a muscle-specific isoform of the  $\gamma$  chain of AMPK. Thus it is provided means to regulate carbohydrate metabolism, more specifically to detect and/or correct potential or actual dysfunctions of the regulation of carbohydrate metabolism, in particular in skeletal muscle.

10 The invention provides a polypeptide comprising an amino acid sequence having at least 70% identity or at least 85% similarity, preferably 80% identity or at least 90% similarity, more preferably at least 90% identity or at least 95% similarity, and still more preferably at least 95% identity or at least 99% similarity, with the polypeptide SEQ ID NO: 2. The invention also provides an isolated nucleic acid sequence encoding said polypeptide, as well as the complement of said nucleic acid sequence.

Said polypeptide represents a new muscle-specific isoform of the  $\gamma$  chain of AMPK, and will also be hereinafter referred as Prkag3; the gene encoding said polypeptide will also be hereinafter referred as PRKAG3.

According to a preferred embodiment of the invention, said polypeptide comprises an amino acid sequence having at least 75% identity, preferably at least 80% identity with the polypeptide SEQ ID NO: 28.

"Identity" of a sequence with a reference sequence refers to the percent of residues that are the same when the two sequences are aligned for maximum correspondence between residues positions. A polypeptide having an amino acid sequence having at least X% identity with a reference sequence is defined herein as a polypeptide whose sequence may include up to 100-X amino acid alterations per each 100 amino acids of the reference amino acid sequence. Amino acids alterations include deletion, substitution or insertion of consecutive or scattered amino acid residues in the reference sequence.

"Similarity" of a sequence with a reference sequence refers to the percent of residues that are the same or only differ by conservative amino acid substitutions when the two sequences are aligned for maximum correspondence between residues positions. A conservative amino acid substitution is defined as the substitution of an amino acid residue for another amino acid residue with similar chemical properties (e.g. size, charge or polarity), which generally does not change the functional properties of the protein. A polypeptide having an amino acid sequence having at least X% similarity with a reference sequence is defined herein as a polypeptide whose sequence may include up to (100-X) non-conservative amino acid alterations per each 100 amino acids of the reference amino acid sequence. Non-conservative amino acids alterations include deletion, insertion, or non-conservative substitution of consecutive or scattered amino acid residues in the reference sequence.

For instance:

\* searching the "GenBank nr" database using BLASTp (ALTSCHUL et al., Nucleic Acids Res., 25, 3389-3402, 1997) with default settings and the whole sequence

SEQ ID NO: 2 as a query, the higher percents of identity or similarity with SEQ ID NO: 2 were found for:

- $\gamma$ 1 subunit of human AMPK: 65% identity or 82% similarity (score: 399);
- 5       -  $\gamma$ 1 subunit of rat AMPK: 65% identity or 82% similarity (score: 399);
- $\gamma$ 1 subunit of murine AMPK: 64% identity or 80% similarity (score: 390);
- $\gamma$  subunit of Drosophila AMPK: 53% identity
- 10     or 75% similarity (score: 332);
- Yeast Snf4: 33% identity or 56% similarity (score: 173);

\* searching the "GenBank nr" database using BLASTp with default settings and the whole sequence

- 15     SEQ ID NO: 28 as a query, the higher percents of identity or similarity were found for:
- $\gamma$ 1 subunit of human AMPK: 64% identity or 80% similarity (score: 403);
  - $\gamma$ 2 subunit of human AMPK: 62% identity or
  - 20     83% similarity (score: 425);
  - $\gamma$ 1 subunit of rat AMPK: 61% identity or 77% similarity (score: 404);
  - $\gamma$ 1 subunit of murine AMPK: 63% identity or 79% similarity (score: 394);
  - 25     -  $\gamma$  subunit of Drosophila AMPK: 52% identity or 76% similarity (score: 340)..

Polypeptides of the invention include for instance any polypeptide (whether natural, synthetic, semi-synthetic, or recombinant) from any vertebrate

30     species, more specifically from birds, such as poultry, or mammals, including bovine, ovine, porcine, murine, equine, and human, and comprising, or consisting of, the amino acid sequence of either:

- a functional Prkag3; or
- 35     - a functionally altered mutant of Prkag3.



"Functional" refers to a protein having a normal biological activity. Such a protein may comprise silent mutations inducing no substantial change in its activity, and having no noticeable phenotypic effects.

5 Non-limitative examples of functional Prkag3 are:

- a porcine Prkag3 comprising at least the sequence represented in the enclosed sequence listing under SEQ ID NO: 2; this includes, for instance the polypeptide SEQ  
10 ID NO: 28;
- a human Prkag3 comprising at least the sequence represented in the enclosed sequence listing under SEQ ID NO: 4; this includes for instance the polypeptide SEQ  
15 ID NO: 30.

The invention also includes splice variants of Prkag3: for instance, the nucleotide sequence SEQ ID NO: 27, and the corresponding amino-acid sequence SEQ ID NO: 28 on one hand, and the nucleotide sequence SEQ ID  
20 NO: 31 and the corresponding amino-acid sequence SEQ ID NO: 32 on the other hand represent two different splice variants of porcine Prkag3.

A "functionally altered mutant" of a protein comprises one or several mutations inducing a change in  
25 its activity. Such mutations include in particular deletions, insertions, or substitutions of amino acid residues in a domain essential for the biological activity of said protein. They may result for instance in a partial or total loss of activity, or conversely in an  
30 increase of activity, or in an impairment of the response to regulatory effectors. Deletions, insertions, or non-conservative substitutions are more likely to result in a critical effect on the biological activity; however conservative substitutions may also induce a noticeable  
35 effect, if they occur at an important position of an active site of the protein.

Non-limitative examples of functionally altered mutants of Prkag3 are:

- the R41Q variant resulting from the non-conservative substitution of an arginine residue in position 41 of SEQ ID NO: 2 or SEQ ID NO: 4 by a glutamine residue (this substitution results in an important increase of the glycogen content, inducing an increased glycolytic potential of the skeletal muscle);
- the V40I variant resulting from the substitution of a valine residue in position 40 of SEQ ID NO: 2 or SEQ ID NO: 4 by an isoleucine residue (this substitution results in a decrease of the glycogen content and thus of the glycolytic potential of the skeletal muscle).

These substitutions occur inside a portion of the first CBS domain that is highly conserved between Prkag3 and the previously known isoforms of the  $\gamma$  subunit of AMPK.

Residue numbers for Prkag3 refer to the amino acid numbering of SEQ ID NO: 2 or SEQ ID NO: 4. Alignment of human and porcine Prkag3 sequences with previously known  $\gamma 1$  and  $\gamma 2$  isoforms is shown in Figure 3.

The invention also provides mutants of Prkag3 which may for instance be obtained by deletion of part of a Prkag3 polypeptide. Said mutants are generally functionally altered. They may have an identity with the overall Prkag3 sequence lower than 70%. However, the identity of the non-deleted sequences of said mutants, when aligned with the corresponding Prkag3 sequences and more specifically with the corresponding sequences from SEQ ID NO: 2, should remain higher than 70%. Said mutants may for instance result from the expression of nucleic acid sequences obtained by deletion or insertion of a nucleic acid segment, or by a punctual mutation introducing a nonsense codon, in a nucleic acid sequence encoding a functional Prkag3.

The invention also provides a functionally altered mutant of a  $\gamma$  subunit of AMPK, wherein said mutant comprises at least one mutation responsible for said functional alteration located within the first CBS domain, and preferably within the region thereof aligned with the region spanning from residue 30 to residue 50 of SEQ ID NO:2 or SEQ ID NO:4. Said mutation may result from the insertion, deletion, and/or substitution of one amino-acid or of several amino-acids, adjacent or not.

More preferably the mutation is located within the region aligned with the region spanning from residue 35 to residue 45 of SEQ ID NO:2 or SEQ ID NO:4, for instance within the region spanning from residue 65 to residue 75 of the  $\gamma 1$  isoform.

According to a particular embodiment, said mutation is a non-conservative substitution, preferably a R $\rightarrow$ Q substitution. According to another particular embodiment, said mutation is a conservative substitution, preferably a V $\rightarrow$ I substitution.

Advantageously, the mutation is located at a residue corresponding to residue 41 of SEQ ID NO:2 or SEQ ID NO:4, for instance in the case of the  $\gamma 1$  isoform, at residue 70, or at a residue corresponding to residue 40 of SEQ ID NO:2 or SEQ ID NO:4, for instance in the case of the  $\gamma 1$  isoform, at residue 69.

The invention also provides a heterotrimeric AMPK wherein the  $\gamma$  subunit consists of a polypeptide of the invention.

The invention also provides isolated nucleic acid sequences encoding any of the above-defined functional or functionally altered Prkag3 or functionally altered mutants of a  $\gamma$  subunit of AMPK, and nucleic acid sequences complementary of any one of these nucleic acid sequences.

This includes particularly any isolated nucleic acid having the sequence of any of the naturally

occurring alleles of a *PRKAG3* gene, as well as any isolated nucleic acid having the sequence of an artificial mutant of a *PRKAG3* gene, provided that said nucleic acid does not consist of the EST GENBANK  
5 AA178898.

This also includes any isolated nucleic acid having the sequence of a natural or artificial mutant of a *PRKAG1* or a *PRKAG2* gene, wherein said mutant encodes a functionally altered  $\gamma 1$  or  $\gamma 2$  subunit of AMPK as defined  
10 above.

Nucleic acids of the invention may be obtained by the well-known methods of recombinant DNA technology and/or of chemical DNA synthesis. These methods also allow to introduce the desired mutations in a naturally  
15 occurring DNA sequence.

Examples of nucleic acids encoding naturally occurring alleles of a *PRKAG3* gene are represented by SEQ ID NO: 1, which encodes a naturally occurring allele of the porcine gene and SEQ ID NO: 3, which encodes a  
20 naturally occurring allele of the human gene. These sequences may be used to generate probes allowing the isolation of *PRKAG3* from other species or of other allelic forms of *PRKAG3* from a same species, by screening a library of genomic DNA or of cDNA.

The invention also includes genomic DNA sequences from any vertebrate species, more specifically from birds, such as poultry, or mammals, including in particular bovine, ovine, porcine, murine, equine, and human, comprising at least a portion of a nucleic acid  
25 sequence encoding a polypeptide of the invention, preferably a portion of a *PRKAG3* gene, and up to 500 kb, preferably up to 100 kb of a 3' and/or of a 5' adjacent genomic sequence.

Such genomic DNA sequences may be obtained by  
35 methods known in the art, for instance by extension of a nucleic acid sequence encoding a polypeptide of the

invention, employing a method such as restriction-site PCR (SARKAR et al., PCR Methods Applic., 2, 318-322, 1993), inverse PCR (TRIGLIA et al., Nucleic Acids Res., 16, 8186, 1988) using divergent primers based on a Prkag3 coding region, capture PCR (LAGERSTROM et al., PCR Methods Applic., 1, 111-119, 1991), or the like.

The invention also includes specific fragments of a nucleic acid sequence encoding a polypeptide of the invention, or of a genomic DNA sequence of the invention as well as nucleic acid fragments specifically hybridising therewith. Preferably these fragments are at least 15bp long, more preferably at least 20bp long.

"Specific fragments" refers to nucleic acid fragments having a sequence that is found only in the nucleic acids sequences encoding a polypeptide of the invention, and is not found in nucleic acids sequences encoding related polypeptides of the prior art. This excludes the nucleic acid fragments that consist of a sequence shared with one of the known PRKAG1 or PRKAG2 genes.

"Specifically hybridising fragments" refers to nucleic acid fragments which can hybridise, under stringent conditions, only with nucleic acid sequences encoding a polypeptide of the invention, without hybridising with nucleic acid sequences encoding related polypeptides of the prior art. This excludes the nucleic acid fragments that consist of the complement of a sequence shared with one of the known PRKAG1 or PRKAG2 genes.

Nucleic acid fragments that consist of the EST GENBANK AA178898 or the EST GENBANK W94830 or the complements thereof are also excluded.

Said specific or specifically hybridising nucleic acid fragments may for example be used as primers or probes for detecting and/or amplifying a nucleic acid sequence encoding a polypeptide of the invention. The

invention encompasses set of primers comprising at least one primer consisting of a specific or specifically hybridising nucleic acid fragment as defined above.

The invention also provides recombinant  
5 vectors comprising a nucleic acid sequence encoding a polypeptide of the invention. Vectors of the invention are preferably expression vectors, wherein a sequence encoding a polypeptide of the invention is placed under control of appropriate transcriptional and translational  
10 control elements. These vectors may be obtained and introduced in a host cell by the well-known recombinant DNA and genetic engineering techniques.

The invention also comprises a prokaryotic or eukaryotic host cell transformed by a vector of the  
15 invention, preferably an expression vector.

A polypeptide of the invention may be obtained by culturing the host cell containing an expression vector comprising a nucleic acid sequence encoding said polypeptide, under conditions suitable for the expression  
20 of the polypeptide, and recovering the polypeptide from the host cell culture.

A heterotrimeric AMPK wherein the  $\gamma$  subunit consists of a polypeptide of the invention may be obtained by expressing, together or separately, a nucleic acid sequence encoding a polypeptide of the invention, a  
25 nucleic acid sequence encoding an  $\alpha$  subunit, and a nucleic acid sequence encoding a  $\beta$  subunit, and reconstituting the heterotrimer.

The polypeptides thus obtained, or immunogenic  
30 fragments thereof may be used to prepare antibodies, employing methods well known in the art. Antibodies directed against the whole Prkag3 polypeptide and able to recognise any variant thereof may thus be obtained. Antibodies directed against a specific epitope of a particular variant (functional or not) of Prkag3 or  
35 antibodies directed against a specific epitope of a

functionally altered mutant having a mutation in the first CBS domain of a  $\gamma$  subunit of AMPK, and able to recognise said variant or functionally altered mutant may also be obtained.

5           As shown herein, mutations in a  $\gamma$  subunit of AMPK, and particularly mutations in the first CBS domain of a  $\gamma$  subunit of AMPK are likely to cause disorders in the energy metabolism (e.g. diabetes, obesity) in vertebrates, including humans. Further, mutations in the  
10 first CBS domain or other parts of the *PRKAG3* gene are likely to cause disorders in the muscular metabolism leading to diseases such as myopathy, diabetes and cardiovascular diseases.

          The present invention provides means for  
15 detecting and correcting said disorders.

          More specifically, the present invention is directed to methods that utilise the nucleic acid sequences and/or polypeptidic sequences of the invention for the diagnostic evaluation, genetic testing and  
20 prognosis of a metabolic disorder.

          For example, the invention provides methods for diagnosing of metabolic disorders, more specifically carbohydrate metabolism disorders, and preferably disorders correlated with an altered, in particular an  
25 excessive, glycogen accumulation in the cells, resulting from a mutation in a gene encoding a  $\gamma$  subunit of AMPK, wherein said methods comprise detecting and/or measuring the expression of a functionally altered *PRKAG3* gene, or of a functionally altered mutant of a  $\gamma$  subunit of AMPK  
30 having a mutation within the first CBS domain in a nucleic acid sample obtained from a vertebrate, or detecting a mutation in the *PRKAG3* gene or in a sequence encoding the first CBS domain of a  $\gamma$  subunit of AMPK in the genome of a vertebrate suspected of having such a  
35 disorder.

According to a preferred embodiment of the invention, the disorder is correlated with an altered, in particular an excessive, glycogen accumulation in the muscular cells and results from the expression of a functionally altered *PRKAG3* gene.

The expression of a functionally altered *Prkag3*, or of a functionally altered mutant of a  $\gamma$  subunit of AMPK having a mutation within the first CBS domain may be detected or measured using either polyclonal or monoclonal antibodies specific for the functionally altered polypeptides of the invention, as defined above. Appropriate methods are known in the art. They include for instance enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS).

The nucleotide sequences of the invention may be used for detecting mutations in the *PRKAG3* gene or in a sequence encoding the first CBS domain of a  $\gamma$  subunit of AMPK, by detection of differences in gene sequences or in adjacent sequences between normal, carrier, or affected individuals.

The invention provides a process for detecting a mutation in the *PRKAG3* gene or in a sequence encoding the first CBS domain of a  $\gamma$  subunit of AMPK wherein said process comprises:

- obtaining a nucleic acid sample from a vertebrate;
- checking the presence in said nucleic acid sample of a nucleic acid sequence encoding a mutant *Prkag3*, or a mutant of a  $\gamma$  subunit of AMPK having a mutation within the first CBS domain, as defined above.

According to a preferred embodiment of the invention there is provided a method for detecting a nucleic acid sequence comprising a mutation in the *PRKAG3* gene or in a sequence encoding the first CBS domain of a  $\gamma$  subunit of AMPK wherein said process comprises:

- obtaining a nucleic acid sample from a vertebrate;



- contacting said nucleic acid sample with a nucleic acid probe obtained from a nucleic acid of the invention and spanning said mutation, under conditions of specific hybridisation between said probe and the mutant sequence to be detected;
- detecting the hybridisation complex.

Preferably, the process of the invention further comprises, prior to hybridisation, PCR amplification from the nucleic acid sample, of a sequence comprising at least the portion of the PRKAG3 sequence or of the sequence encoding the first CBS domain of the  $\gamma$  subunit of AMPK wherein the mutation is to be detected.

Methods allowing the specific hybridisation of a probe only with a perfectly matching complementary sequence, and useful for the detection of punctual mutations are known in the art. They include for instance Allele Specific PCR (GIBBS, Nucleic Acid Res., 17, 2427-2448, 1989), Allele Specific Oligonucleotide Screening (SAIKI et al., Nature, 324, 163-166, 1986), and the like.

A mutation in the PRKAG3 gene may also be detected through detection of polymorphic markers closely linked to said mutation.

The invention also provides means for identifying said polymorphic markers, and more specifically polymorphic markers comprised within a genomic DNA sequence comprising at least a portion of a PRKAG3 gene, and up to 500 kb, preferably 300 kb, more preferably up to 100 kb of a 3' and/or of a 5' adjacent sequence.

Said polymorphic markers may be obtained for instance, by screening a genomic DNA library from a vertebrate with a probe specific for the PRKAG3 gene, in order to select clones comprising said nucleic acid sequence and flanking chromosomal sequences, and identifying a polymorphic marker in said flanking chromosomal sequences. The allele(s) of a polymorphic

marker associated with a given mutant allele of the *PRKAG3* gene may also easily be identified by use of a genomic DNA library from an individual wherein the presence of said mutant allele has previously been  
5 detected by hybridisation with a nucleic acid probe of the invention.

Polymorphic markers include for instance, single nucleotide polymorphisms (SNP), microsatellites, insertion/deletion polymorphism and restriction fragment  
10 length polymorphism (RFLP). These polymorphic markers may be identified by comparison of sequences flanking the *PRKAG3* gene obtained from several individuals. Microsatellites may also be identified by hybridisation with a nucleic acid probe specific of known  
15 microsatellite motifs.

Once a polymorphic marker has been identified, a DNA segment spanning the polymorphic locus may be sequenced and a set of primers allowing amplification of said DNA segment may be designed.

20 The invention also encompasses said DNA primers.

Detection of a mutation in the *PRKAG3* gene may be performed by obtaining a sample of genomic DNA from a vertebrate, amplifying a segment of said DNA spanning a  
25 polymorphic marker by polymerase chain reaction using a set of primers of the invention, and detecting in said amplified DNA the presence of an allele of said polymorphic marker associated with said mutation.

By way of example, polymorphic markers which  
30 may be obtained according to the invention, and DNA primers allowing the detection of polymorphic markers closely linked to the *RN* allele of porcine *PRKAG3* gene are listed in Table 1 hereinafter.

According to a preferred embodiment of the  
35 invention, the vertebrate is a mammal, preferably a farm animal and more preferably a porcine, and the mutation to

be detected produces a functionally altered Prkag3. The detection of said mutation allows to predict whether said mammal or the progeny thereof is likely to have an intramuscular glycogen concentration higher or lower than the average. An example of such a mutation produces a functionally altered Prkag3 having a R41Q substitution, and resulting in an increased glycogen content in the skeletal muscle.

Another example of such a mutation produces a functionally altered Prkag3 having a V40I substitution, and resulting in a decreased glycogen content in the skeletal muscle. In farm animals having such a mutation, glycogenolysis which occurs after slaughtering is less important than in normal animals, resulting in a higher pH and in a potential better quality of the meat.

The present invention also includes kits for the practice of the methods of the invention. The kits comprise any container which contains at least one specific fragment of a nucleic acid sequence of the invention, or at least one nucleic acid fragment able to specifically hybridise with a nucleic acid sequence of the invention. Said nucleic acid fragment may be labelled. The kits may also comprise a set of primers of the invention. They may be used in conjunction with commercially available amplification kits. They may also include positive or negative control reactions or markers, molecular weight size markers for gel electrophoresis, and the like.

Other kits of the invention may include antibodies of the invention, optionally labelled, as well as the appropriate reagents for detecting an antigen-antibody reaction. They may also include positive or negative control reactions or markers.

The invention further provides means for modulating the expression of vertebrate genes encoding a  $\gamma$  subunit of AMPK, and more specifically of the PRKAG3 gene

and/or the synthesis or activity of the products of said genes.

A purified AMPK heterotrimer comprising wild-type or mutant Prkag3 subunit, or a functionally altered mutant  $\gamma$  subunit having a mutation in the first CBS domain, may be used for screening *in vitro* compounds able to modulate AMPK activity, or to restore altered AMPK activity. This may be done, for instance, by:

- measuring the binding of the compound to said heterotrimer, using for example high-throughput screening methods; or,

- measuring changes in AMPK kinase activity, using for example high-throughput screening methods.

High throughput screening methods are disclosed, for instance, in "High throughput screening: The Discovery of Bioactive Substances", J.P. DEVLIN (Ed), MARCEL DEKKER Inc., New York (1997).

Nucleic acids of the invention may be used for therapeutic purposes. For instance, complementary molecules or fragments thereof (antisense oligonucleotides) may be used to modulate AMPK activity, more specifically in muscular tissue.

Also, a nucleic acid sequence encoding a functional Prkag3 may be used for restoring a normal AMPK function.

Transformed cells or animal tissues expressing a wild-type or mutant Prkag3, or a functionally altered mutant of a  $\gamma$  subunit of AMPK as defined above, or expressing an AMPK comprising said mutant Prkag3, or said functionally altered mutant of a  $\gamma$  subunit of AMPK, may be used as *in vitro* model for elucidating the mechanism of AMPK activity or for screening compounds able to modulate the expression of AMPK.

The screening may be performed by adding the compound to be tested to the culture medium of said cells or said tissues, and measuring alterations in energy

metabolism in said cells or said tissues using methods such as measurements of glucose concentrations (levels), glucose uptake, or changes of the ATP/AMP ratio, glycogen or lipid/protein content..

5           The invention provides animals transformed with a nucleic acid sequence of the invention.

          In one embodiment, said animals are transgenic animals having at least a transgene comprising a nucleic acid of the invention.

10           In another embodiment, said animals are knockout animals. "Knockout animals" refers to animals whose native or endogenous *PRKAG3* alleles have been inactivated and which produce no functional *Prkag3* of their own.

15           In light of the disclosure of the invention of DNA sequences encoding a wild-type or mutant *Prkag3*, or a functionally altered mutant of a  $\gamma$  subunit of AMPK, transgenic animals as well as knockout animals may be produced in accordance with techniques known in the art,  
20   for instance by means of *in vivo* homologous recombination.

          Suitable methods for the preparation of transgenic or knock-out animals are for instance disclosed in: *Manipulating the Mouse Embryo*, 2<sup>nd</sup> Ed., by  
25   HOGAN et al., Cold Spring Harbor Laboratory Press, 1994; *Transgenic Animal Technology*, edited by C. PINKERT, Academic Press Inc., 1994; *Gene Targeting: A Practical Approach*, edited by A.L. JOYNER, Oxford University Press, 1995; *Strategies in Transgenic Animal Science*, edited by  
30   G.M. MONASTERSKY and J.M. ROBL, ASM Press, 1995; *Mouse Genetics: Concepts and Applications*, by Lee M. SILVER, Oxford University Press, 1995.

          These animals may be used as models for metabolic diseases and disorders, more specifically for  
35   diseases and disorders of glycogen metabolism in muscle. For instance they may be used for screening test

molecules. Transgenic animals may thus be used for screening compounds able to modulate AMPK activity. Knockout animals of the invention may be used, in particular, for screening compounds able to modulate energy metabolism, more specifically carbohydrate metabolism, in the absence of functional Prkag3.

The screening may be performed by administering the compound to be tested to the animal, and measuring alterations in energy metabolism in said animal using methods such as glucose tolerance tests, measurements of insulin levels in blood, changes of the ATP/AMP ratio, glycogen or lipid/protein content in tissues and cells.

Transgenic or knock-out farm animals with modified meat characteristics or modified energy metabolism may also be obtained.

The present invention will be further illustrated by the additional description which follows, which refers to examples of obtention and use of nucleic acids of the invention. It should be understood however that these examples are given only by way of illustration of the invention and do not constitute in any way a limitation thereof.

#### **EXAMPLE 1: ISOLATING THE PRKAG3 GENE**

We have screened a porcine Bacterial Artificial Chromosome (BAC) library (ROGEL-GAILLARD et al., Cytogenet and Cell Genet, 851, 273-278, 1999) and constructed a contig of overlapping BAC clones across the region of pig chromosome 15 harbouring the RN gene. These BAC clones were in turn used to develop new genetic markers in the form of single nucleotide polymorphisms (SNPs) or microsatellites (MS) as described in Table 1 below.

Table 1

|    | Name of marker | BAC clone                             | Primer sequences   | Size of PCR product (bp) | Marker type <sup>a</sup> | Alleles <sup>b</sup>                 |
|----|----------------|---------------------------------------|--|--------------------------|--------------------------|--------------------------------------|
| 1  | H3             | 115B9, 156E6, 361B4, 90A9             | F: 5'-GGAATTTCAAGTCAGCCAAC-3' (SEQ ID NO: 5)<br>R: 5'-CTTCAAAAGACCGTGCTACT-3' (SEQ ID NO: 6)       | 114 - 138                | MS                       | 114, 126, 128, 132*, 134*, 136, 138  |
| 2  | MS982H1        | 982H11                                | F: 5'-CTGGGAACCTCTATATGCTG-3' (SEQ ID NO: 7)<br>R: 5'-TAGGGAATACAAATCACAG-3' (SEQ ID NO: 8)        | 114 - 157                | MS                       | 114, 140, 142*, 144, 146, 150, 158   |
| 3  | MS479L3        | 479L3, 297D7, 852B5, 153B5            | F: 5'-CTCCAGCTCACAGGATGACA-3' (SEQ ID NO: 9)<br>R: 5'-GTTCTGCAGCTTAGCATCTATTCC-3' (SEQ ID NO: 10)  | 150 - 164                | MS                       | 150*, 160, 162, 164                  |
| 4  | MS997M3        | 997F12                                | F: 5'-GAAGTATCCTGGCTTCTGA-3' (SEQ ID NO: 11)<br>R: 5'-GTTCTCCAGGTTCCAGACATCCAC-3' (SEQ ID NO: 12)  | 138 - 160                | MS                       | 138, 144, 152, 154, 160*             |
| 5  | MS482H6        | 482E7                                 | F: 5'-GCTTCTGCTGCCCTACTT-3' (SEQ ID NO: 13)<br>R: 5'-GTTCTAAGTTCTACTGTAAGACACCC-3' (SEQ ID NO: 14) | 78 - 90                  | MS                       | 78, 80, 88*, 90                      |
| 6  | MS337H2        | 808G10, 947E5, 337G11                 | F: 5'-CCAAGCTGTGGTGGCTGAAT-3' (SEQ ID NO: 15)<br>R: 5'-CAGCACAGCAGTGCCACCTA-3' (SEQ ID NO: 16)     | 145 - 165                | MS                       | 145, 149, 155, 161*, 165*            |
| 7  | MS127B1        | 127G6, 134C9                          | F: 5'-CAAACCTTCTAGGCGTGT-3' (SEQ ID NO: 17)<br>R: 5'-GTTCTGGAACCTCCATATGCCATGG-3' (SEQ ID NO: 18)  | 94 - 108                 | MS                       | 94, 100, 108*, 114                   |
| 8  | CMKAR2         | 128A3, 337G11, 808G10, 947E7, 1110H12 | F: 5'-AGGGTGGATGGTAGGCTTCA-3' (SEQ ID NO: 19)<br>R: 5'-GTCTCGCTCCTGAAGGAAGT-3' (SEQ ID NO: 20)     | 208                      | SNP                      | 112A*, 112T, 158A*, 158G 176A*, 176G |
| 9  | 127G63         | 127G6, 134C9, 170D7, 1030A5, 1088F2   | F: 5'-AGTCACGTGGCCATGCTATC-3' (SEQ ID NO: 21)<br>R: 5'-CTCAACTGGATTGAGTCAGT-3' (SEQ ID NO: 22)     | 409                      | SNP                      | 234A*, 234C                          |
| 10 | VIL1           | 1088F2                                | F: 5'-TTGGCGCAACTGTTATTCT-3' (SEQ ID NO: 23)<br>R: 5'-AGGCAAGGAAGAGACACAG-3' (SEQ ID NO: 24)       | 270                      | SNP                      | 90T, 90G, 120A, 120G, 166C, 166T     |
| 11 | NRAMP1         | 315F7, 530A6, 651C12, 1088F2, 1095H3  | F: 5'-AGCCGTGGGCATCGTTGG-3' (SEQ ID NO: 25)<br>R: 5'-AGAAGGAGACAGACAGGGCGA-3' (SEQ ID NO: 26)      | 1300                     | RFLP (Syl)               | 1: 100+1200 bp<br>2: 100+200+1000 bp |

<sup>a</sup>MS=microsatellite; SNP=single nucleotide polymorphism.<sup>b</sup>Microsatellite alleles are designated according to the length of the amplified fragment while SNPs are denoted according to the polymorphic nucleotide. Alleles associated with the RFLP allele are marked with an asterisk.

The new markers were used together with some previously described markers to construct a high-resolution linkage map. Standard linkage analysis using pedigree data comprising about 1,000 informative meioses for segregation at the RN locus made it possible to exclude RN from the region proximal to MS479L3 and distal to microsatellite Sw936. Linkage Disequilibrium (LD) analysis was done with the same markers and a random sample of 68 breeding boars from the Swedish Hampshire population, scored for the RN phenotype by measuring glycogen content in muscle. The results of LD analysis using the DISMULT program (TERWILLIGER, Am. J. Hum. Genet., 56, 777-787, 1995) are shown in Figure 1. They reveal a sharp LD peak around the markers MS127B1 and SNP127G63. These markers appeared to show complete linkage disequilibrium with the RN allele, i.e. RN was associated with a single allele at these two loci. The most simple interpretation of this finding is that the RN mutation arose on a chromosome carrying these alleles and that the two markers are so closely linked to the RN locus that the recombination frequency is close to 0%. The two markers are both present on the overlapping BAC clones 127G6 and 134C9 suggesting that the RN gene may reside on the same clone or one of the neighbouring clones.

A shot-gun library of the BAC clone 127G6 was constructed and more than 1,000 sequence reads were collected giving about 500,000 base pair random DNA sequence from the clone. The data were analysed and sequence contigs constructed with the PHRED, PHRAP and CONSED software package (University of Washington Genome Center, <http://bozeman.mbt.washington.edu>). The sequence data were masked for repeats using the REPEATMASKER software (<http://ftp.genome.washington.edu/cgi-bin/RepeatMasker>) and BLAST searches were carried out using the NCBI web site (<http://www.ncbi.nlm.nih.gov>).



Three convincing matches to coding sequences were obtained. Two of these were against human cDNA sequences/genes, KIAA0173 described as being similar to pig tubulin-tyrosine ligase and located on HSA2q (UniGene cluster Hs.169910, <http://www.ncbi.nlm.nih.gov/UniGene/>) and CYP27A1 located on HSA2q33-ter (UniGene cluster Hs.82568). The results strongly suggested that the pig coding sequences are orthologous to these human genes as it is well established that the RN region is homologous to HSA2q33-36 (ROBIC et al., Mamm. Genome, 10, 565-568, 1999). However, none of these sequences appeared as plausible candidate genes for RN. The third coding sequence identified in BAC 127G6 showed highly significant sequence similarity to various AMP-activated protein kinase  $\gamma$  sequences including the yeast SNF4 sequence. The cDNA sequence of this gene was determined by RT-PCR and RACE analysis using muscle mRNA from an  $rn^+/rn^+$  homozygote. This sequence is shown in Figure 2 and in the enclosed sequence listing under SEQ ID NO: 1.

Legend of Figure 2:  
5' UTR: 5' untranslated region  
3' UTR: 3' untranslated region  
CDS: coding sequence  
\*\*\*: stop codon  
'-': identity to master sequence  
'.': alignment gap

The frame of translation was determined on the basis of homology to other members in the protein family and assuming that the first methionine codon in frame is the start codon. The polypeptidic sequence deduced on this basis is shown in the enclosed sequence listing under SEQ ID NO: 2.

The complete nucleotidic sequence of pig PRKAG3 cDNA is shown in the enclosed sequence listing under SEQ ID NO: 27 and the complete polypeptidic

sequence is shown in the enclosed sequence listing under SEQ ID NO: 28 and in Figure 3.

Figure 3 shows an amino acid alignment constructed with the CLUSTAL W program (THOMPSON et al.,  
 5 Nucleic Acids Research, 22, 4673-4680, 1994) with representative AMPK  $\gamma$  sequences in the nucleotide databases.

#### Legend of Figure 3:

##### Sequences used:

- 10 HumG1: Genbank U42412
  - MusG1: Genbank AF036535
  - HumG2: Human PRKAG2 (Genbank AJ249976)
  - PigG3: pig PRKAG3 (this study)
  - HumG3: human PRKAG3 (this study)
  - 15 Dros: *Drosophila* (Genbank AF094764)
  - SNF4 (yeast): Genbank M30470
- Both the PRKAG2 and *Drosophila* sequences have longer aminoterminal regions but they do not show significant homology to the aminoterminal region of PRKAG3 and were  
 20 not included.

##### Abbreviations:

- \*: stop codon
  - '-': identity to master sequence
  - '..': alignment gap
- 25 The four CBS domains are overlined and the position of the RN<sup>o</sup> mutation is indicated by an arrow.

Table 2 below shows the amino acid (above diagonal) and nucleotide sequence (below diagonal) identities (in %) among mammalian, *Drosophila* and yeast  
 30 AMPKG/SNF4 sequences. In the case of pig PRKAG3 and human PRKAG3, the identities were calculated referring to the portions thereof represented respectively by SEQ ID NO: 1 and SEQ ID NO: 3, for the nucleotide sequences, and by SEQ ID NO: 2 and SEQ ID NO: 4, for the amino acid  
 35 sequences.

TABLE 2

|       | PigG3 | HumG3 | HumG1 | RatG1 | MusG1 | HumG2 | Dros | SNF4 |
|-------|-------|-------|-------|-------|-------|-------|------|------|
| PigG3 | -     | 97.0  | 64.2  | 64.2  | 63.9  | 62.6  | 53.2 | 34.0 |
| HumG3 | 90.7  | -     | 63.6  | 63.6  | 63.6  | 62.6  | 53.5 | 34.4 |
| HumG1 | 64.2  | 64.5  | -     | 96.7  | 96.3  | 75.6  | 60.9 | 33.5 |
| RatG1 | 65.8  | 65.8  | 88.0  | -     | 97.4  | 75.3  | 61.1 | 33.5 |
| MusG1 | 65.3  | 64.8  | 87.2  | 92.8  | -     | 74.6  | 61.7 | 33.5 |
| HumG2 | 61.6  | 61.6  | 68.1  | 67.8  | 65.9  | -     | 63.1 | 34.5 |
| Dros  | 58.4  | 58.4  | 59.0  | 59.3  | 59.0  | 60.0  | -    | 36.2 |
| SNF4  | 44.0  | 44.2  | 45.4  | 44.6  | 45.3  | 45.7  | 44.8 | -    |

Figure 4 shows a Neighbor-Joining phylogenetic tree constructed with the PAUP software (SWOFFORD, *Phylogenetic analysis using parsimony (and other methods)*, Sinauer Associates, Inc. Publishers, Sunderland, Massachusetts, 1998) using yeast SNF4 as outgroup; support for branch orders obtained in bootstrap analysis with 1,000 replicates are indicated, scales of tree is indicated at the bottom. The result showed that the pig gene located in the RN region is distinct from mammalian PRKAG1 and PRKAG2 isoforms and most likely orthologous to a human gene represented by the human EST sequence AA178898 (GenBank) derived from a muscle cDNA library. This gene is herein denoted PRKAG3 since it is the third isoform of a mammalian AMP-activated protein kinase  $\gamma$  characterised so far.

The cDNA sequence of this gene was determined by RT-PCR and 5'RACE analysis using human skeletal muscle cDNA (Clontech, Palo Alto, CA). This sequence is shown in Figure 2 and in the sequence listing under SEQ ID NO: 3. The deduced polypeptidic sequence having 97% identity with the porcine sequence SEQ ID NO: 2 (cf. Table 2) is shown on Figure 2 and in the sequence listing under SEQ ID NO: 4.

The complete cDNA sequence is also shown in the enclosed sequence listing under SEQ ID NO: 29; the deduced polypeptidic sequence is shown in the enclosed sequence listing under SEQ ID NO: 30 and in Figure 3.

Using the high resolution human TNG radiation hybrid panel : (<http://shgc-www.stanford.edu/RH/TNGindex.html>) we mapped the human homologs of *PRKAG3*, *CYP27A1* and *KIAA0173*, all present in the porcine BAC127G6. The three  
5 genes are also very closely linked in the human genome. *PRKAG3* was mapped at a distance of 33 cR<sub>50.000</sub> from *KIAA0173* and 52 cR<sub>50.000</sub> from *CYP27A1*, with lod score support of 6.8 and 4.5, respectively.

The established role of AMPK in regulating  
10 energy metabolism, including glycogen storage, and its location in the region showing maximum linkage disequilibrium made *PRKAG3* a very strong candidate gene for RN. This was further strengthened by hybridisation analysis of a human multiple tissue northern blots  
15 (CLONTECH, Palo Alto, CA) using human *PRKAG1* (IMAGE clone 0362755 corresponding to GenBank entry AA018675), human *PRKAG2* (IMAGE clone 0322735 corresponding to GenBank entry W15439) and a porcine *PRKAG3* probe. The results are shown in Figure 5.

20 Legend of Figure 5:

H: Heart, B: Brain, Pl: Placenta, L: Lung,  
Li: Liver, M: Skeletal muscle, K: Kidney, Pa: Pancreas,  
S: Spleen, Th: Thymus, P: Prostate, T: Testis, O: Ovary,  
I: Small intestine, C: Colon (mucosal lining),  
25 PBL: Peripheral Blood Leukocyte.

While the *PRKAG1* and *PRKAG2* probes showed a broad tissue distribution of expression, *PRKAG3* showed a distinct muscle-specific expression. This result is also supported by the human EST database where multiple ESTs  
30 representing *PRKAG1* and *PRKAG2* have been identified in various cDNA libraries whereas a single EST (GenBank entry AA178898) representing *PRKAG3* has been obtained from a muscle cDNA library. The muscle-specific expression of *PRKAG3* and the lack of expression in liver  
35 are entirely consistent with the phenotypic effect of RN, namely that glycogen content is altered in muscle but

normal in liver (ESTRADE et al., Comp. Biochem. Physiol. 104B, 321-326, 1993).

*PRKAG3* sequences were determined from *rn<sup>+</sup>/rn<sup>+</sup>* and *RN<sup>-</sup>/RN<sup>-</sup>* homozygotes by RT-PCR analysis. A comparison  
5 revealed a total of seven nucleotide differences four of which were nonsynonymous substitutions was found between the sequence from *rn<sup>+</sup>* and *RN<sup>-</sup>* animals, as shown in Table 3 below. Screening of these seven SNPs with genomic DNA from additional *rn<sup>+</sup>* and *RN<sup>-</sup>* pigs of different breeds  
10 revealed five different *PRKAG3* alleles, but only the R41Q missense substitution was exclusively associated with *RN<sup>-</sup>*. This nonconservative substitution occurs in CBS1 which is the most conserved region among isotypic forms of the AMPK  $\gamma$  chain and arginine at this residue (number 70 in  
15 *Prkag1*) is conserved among different isoforms of mammalian AMPK  $\gamma$  sequences as well as in the corresponding *Drosophila* sequence (Figure 3). A simple diagnostic DNA test for the R41Q mutation was designed based on the oligonucleotide ligation assay (OLA; LANDEGREN et al.,  
20 Science, 241, 1077-1080, 1988). Screening a large number of *RN<sup>-</sup>* and *rn<sup>+</sup>* animals from the Hampshire breed as well as large number of *rn<sup>+</sup>* animals from other breeds showed that the 41Q allele was present in all *RN<sup>-</sup>* animals but not found in any *rn<sup>+</sup>* animals, as shown in Table 4 below. The  
25 absence of the 41Q allele from other breeds is consistent with the assumption that the *RN<sup>-</sup>* allele originated in the Hampshire breed; the allele has not yet been found in purebred animals from other breeds. In conclusion, the results provide convincing evidence that *PRKAG3* is  
30 identical to the *RN* gene and that the R41Q substitution most likely is the causative mutation.

Table 3. Comparison of the *PRKAG3* sequences associated with the *rn*<sup>+</sup> and *RN* alleles in different pig populations<sup>a</sup>

| Associated<br>allele   | RN allele | nt83 | nt152 | Codon |     |     |     |     | Population <sup>b</sup> |
|------------------------|-----------|------|-------|-------|-----|-----|-----|-----|-------------------------|
|                        |           |      |       | 34    | 35  | 40  | 41  | 213 |                         |
| <i>RN</i>              |           | ACC  | CTC   | GCC   | CTG | GTC | CAA | TCT | H                       |
|                        |           | T    | L     | A     | L   | V   | Q   | S   |                         |
| <i>rn</i> <sup>+</sup> |           | ---  | ---   | ---   | --- | --- | -G- | --- | L, LW, WB               |
|                        |           | -    | -     | -     | -   | -   | R   | -   |                         |
| <i>rn</i> <sup>+</sup> |           | ---  | -C-   | --T   | T-- | --- | -G- | --C | H, L, LW, M, WB         |
|                        |           | -    | P     | -     | -   | -   | R   | -   |                         |
| <i>rn</i> <sup>+</sup> |           | -A-  | -C-   | --T   | T-- | --- | -G- | --C | D, H                    |
|                        |           | N    | P     | -     | -   | -   | R   | -   |                         |
| <i>rn</i> <sup>+</sup> |           | ---  | -C-   | --T   | T-- | A-- | -G- | --C | H, LW, WB, D, L         |
|                        |           | -    | P     | -     | -   | I   | R   | -   |                         |

nucleotide and codon numbers refer to the numbering of the sequence SEQ ID NO: 1

i=Hampshire, L=Landrace, LW=Large White, M=Meishan, WB=Wild Boar, D=Duroc

.D.=not determined, "-" indicates identity to the top sequence.

TABLE 4

| RN phenotype                                | Genotype at nucleotide 593 <sup>d</sup> |     |     | Total |
|---|---|-----|-----|-------|
|   | A/A                                     | G/A | G/G |       |
| RN <sup>-</sup> , Hampshire <sup>a</sup>    | 40                                      | 87  | 0   | 127   |
| RN <sup>-</sup> , Hampshire <sup>a,b</sup>  | 0                                       | 13  | 0   | 13    |
| rn <sup>+</sup> , Hampshire <sup>a</sup>    | 0                                       | 0   | 60  | 60    |
| rn <sup>+</sup> , other breeds <sup>c</sup> | 0                                       | 0   | 488 | 488   |

<sup>a</sup>represent both French and Swedish Hampshire populations

<sup>b</sup>heterozygosity RN/rn<sup>+</sup> deduced using pedigree information

<sup>c</sup>breeds: Angler Saddleback, n=31; Blond Mangalitza, n=2; Bunte Bentheimer, n=16; Duroc, n=160; Göttinger Minipig, n=4; Landrace, n=83; Large White, n=72; Meishan, n=8; Piétrain, n=75; Red Mangalitza, n=5; Rotbunte Husumer, n=15; Schwalbenbauch Mangalitza, n=7; Schwäbisch Hällische, n=2; European Wild Boar, n=5; Japanese Wild Boar, n=3.

<sup>d</sup>refers to the nucleotide numbers of SEQ ID NO: 1

Without being bound to any particular mechanism, it may be hypothesised that the AMPK heterotrimer including PRKAG3 is involved in the regulation of glucose transport into skeletal muscle.

It has recently been reported that AMPK activation induced by the AMP analogue AICAR or by muscle contraction leads to an increased glucose uptake in skeletal muscle (BERGERON et al., Am. J. Physiol., 276, E938-944, 1999; HAYASHI et al., Diabetes, 47, 1369-1373, 1998). If this is the function of the AMPK heterotrimer including PRKAG3, R41Q may be a gain-of-function mutation causing a constitutively active holoenzyme, for instance due to the loss of an inactivating allosteric site. If so, the reduced AMPK activity in RN<sup>-</sup> animals is likely to reflect feed-back inhibition due to the high-energy status of the muscle. An increased uptake of glucose to skeletal muscle is expected to lead to an increase in muscle glycogen content as observed in RN<sup>-</sup> animals. It has been shown that overexpression of glucose transporter 4 (GLUT4) in transgenic mice leads to increased uptake of glucose and increased glycogen storage (TREADWAY et al., J. Biol. Chem., 269, 29956-29961, 1994). This type of gain-of-function model is consistent with the dominance

of *RN* as the presence of a single unregulated copy would have a large effect on AMPK enzyme activity.

An alternative hypothesis on the functional significance of the R41Q substitution associated with the *RN* allele may also be proposed. Based on the established roles of the yeast SNF1 enzyme in utilisation of glycogen and of mammalian AMPK for inhibiting energy-consuming pathways and stimulating energy-producing pathways, activated AMPK is expected to inhibit glycogen synthesis and stimulate glycogen degradation. If this is the functional role of the isoform(s) containing the *PRKAG3* product, the R41Q substitution would be a loss-of-function mutation or a dominant-negative mutation locking the AMPK heterotrimer in an inactive state, and thus inhibiting AMP activation and glycogen degradation. In these cases the phenotypic effect should be explained by haplo-insufficiency, since *RN* appears fully dominant.

R41Q may thus be a dominant negative mutation, but only if it interferes with multiple isoforms since the major AMPK activity in muscle appears to be associated with the *PRKAG1* and 2 isoforms [CHEUNG, et al. *Biochem. J.* 346, 659 (2000)].

The distinct phenotype of the *RN* mutation indicates that *PRKAG3* plays a key role in the regulation of energy metabolism in skeletal muscle. For instance, *PRKAG3* is likely to be involved in the adaptation to physical exercise, which is associated with increased glycogen storage. It is also conceivable that loss-of-function mutations in *PRKAG3* (or other AMPK genes) may predispose individuals to noninsulin-dependent diabetes mellitus, and AMPK isoforms are potential drug targets for treatment of this disorder.

#### EXAMPLE 2: DETECTION OF THE R41Q SUBSTITUTION IN PIG *PRKAG3*

A part of *PRKAG3* including codon 41 was amplified in 10 µl reactions containing 100 ng genomic



DNA, 0.2 mM dNTPs, 1.5 mM MgCl<sub>2</sub>, 4.0 pmol of both forward (AMPKG3F3:5'-GGAGCAAATGTGCAGACAAG-3') and reverse (AMPKG3R2:5'-CCCACGAAGCTCTGCTTCTT-3') primer, 10% DMSO, 1 U of Taq DNA polymerase and reaction buffer (ADVANCED BIOTECH, London, UK). The cycling conditions included an initial incubation at 94°C for 5 min followed by 3 cycles at 94°C (1 min), 57°C (1 min) and 72°C (1 min), and 35 cycles of 94°C (20 sec), 55°C (30 sec) and 72°C (30 sec). Allele discrimination at nucleotide position 122 was done using the oligonucleotide ligation assay (OLA, LANDEGREN et al., Science, 241, 1077-1080, 1988). The OLA method was carried out as a gel-based assay. Each 10 µl OLA reaction contained 0.5 pmol of each probe SNPRN-A (5'-Hex-TGGCCAACGGCGTCCA-3'), SNPRN-G (5'-ROX-GGCCAACGGCGTCCG-3') and SNPRN-Common (5'-phosphate-AGCGGCACCTTTGTGAAAAAAAAA-3'), 1.5 U of thermostable AMPLIGASE and reaction buffer (EPICENTRE TECHNOLOGIES, Madison, WI) and 0.5 µl of the AMPKG3F3/AMPKG3R2 PCR product. After an initial incubation at 95°C for 5 min, the following thermocycling profile was repeated 10 times: denaturation at 94°C (30 sec), and probe annealing and ligation at 55°C (90 sec). After OLA cycling, 1 µl of product was heat denatured at 94°C (3 min), cooled on ice, and loaded onto 6% polyacrylamide denaturing gel for electrophoresis on an ABI377 DNA sequencer (PERKIN ELMER, Foster City, USA). The resulting fragment lengths and peak fluorescence were analysed using GENESCAN software (PERKIN ELMER, Foster City, USA).

The OLA-based method for the R41Q mutation was used to determine the genotype of DNA samples collected from 68 Swedish Hampshire animals phenotyped as either RN<sup>-</sup> or rn<sup>+</sup> based on their glycolytic potential (GP) value. Figure 6 illustrates typical OLA results from the three possible genotypes. All RN<sup>-</sup> animals were scored as homozygous A/A (n=28) or heterozygous A/G (n=36) at

nucleotide position 122 whereas the  $rn^+$  animals were homozygous G/G ( $n=4$ ) at this position.

**EXAMPLE 3: PREDICTING THE PRESENCE OF THE  $RN^-$  ALLELE USING A CLOSELY LINKED MICROSATELLITE, MS127B1**

5        A microsatellite 127B1 (MS127B1) was cloned from BAC 127G7 containing pig *PRKAG3*. The BAC clone was digested with *Sau3AI* and the restriction fragments subcloned into the *Bam*HI site of pUC18. The resulting library was probed with a  $(CA)_{15}$  oligonucleotide probe labelled with  $[\gamma-^{32}P]$ -  
10        dATP. Strongly hybridising clones were sequenced and primers for PCR amplification of microsatellite loci were designed. Ten  $\mu$ l PCR reactions were performed containing 100 ng genomic DNA, 0.2 mM dNTPs, 1.5 mM  $MgCl_2$ , 4.0 pmol of both forward (MS127B1F:5'-Fluorescein-  
15        CAAACTCTTCTAGGCGTGT-3') and reverse (MS127B1R:5'-GTTTCTGGAACTTCCATATGCCATGG-3') primers, and 1 U of *Taq* DNA polymerase and reaction buffer (ADVANCED BIOTECH, London, UK). The cycling conditions included an initial incubation at 94°C for 5 min followed by 3 cycles at 94°C  
20        (1 min), 57°C (1 min) and 72°C (1 min), and 35 cycles of 94°C (20 sec), 55°C (30 sec) and 72°C (30 sec). The PCR products (0.3  $\mu$ l) were separated using 4% polyacrylamide denaturing gel electrophoresis on an ABI377 DNA sequencer (PERKIN ELMER, Foster City, USA). The resulting fragment  
25        lengths were analysed using the GENESCAN and GENOTYPER software (PERKIN ELMER, Foster City, USA).

30        The method was used to determine the genotype of DNA samples collected from 87 Swedish Hampshire animals phenotyped as either  $RN^-$  or  $rn^+$  based on their glycolytic potential (GP) value. Allele 108 (bp) showed a complete association to the  $RN^-$  allele in this material as all  $RN^-$  ( $RN^-/RN^-$  or  $RN^-/rn^+$ ) animals were homozygous or heterozygous for this allele while no  $rn^+$  ( $rn^+/rn^+$ ) animals carried this allele, as shown in Table 5 below.

TABLE 5

| Animals         | n  | Genotype |        |        |         |         |
|-----------------|----|----------|--------|--------|---------|---------|
|                 |    | 94/94    | 94/108 | 94/114 | 100/108 | 108/108 |
| RN <sup>-</sup> | 80 | 0        | 37     | 0      | 2       | 41      |
| rn <sup>+</sup> | 7  | 3        | 0      | 4      | 0       | 0       |

**EXAMPLE 4: DETECTING THE PRESENCE OF THE RN<sup>-</sup> ALLELE USING A PCR-RFLP TEST**

The RN<sup>-</sup> mutation inactivates a BsrBI site  
 5 GAG<sup>+</sup>CGG/CTC<sup>+</sup>GCC (BsrBI RE site is not palindromic). At that site, the RN<sup>-</sup> sequence is AAGCGG instead of GAGCGG.

A 134 bp long fragment of the RN gene is amplified from porcine genomic DNA. The rn<sup>+</sup> allele is identified after BsrBI digestion, by detection of two  
 10 fragments of 83 and 51 bps.

The test is performed as follows:

1° Primer sequences:

Sequence of primers used to amplify the RN mutation region:

15 RNU: 5' GGAACGATTCACCCTCAAC 3'

RNL: 5' AGCCCTCCTCACCACGAA 3'

To provide an internal control of digestion, a BsrBI site has been added at the extremity of one of the two primers within a 20 bp long tail. The tail permits  
 20 both creation of a BsrBI site (a shorter tail might be sufficient), and an easy discrimination of uncut fragment from other fragments. The use of tailed primers does not affect efficiency and specificity of amplification.

The sequence of the RNL modified primer  
 25 including a control tail with a BsrBI site is:

RNLBsrA14: 5'

A<sub>5</sub>C<sub>2</sub>A<sub>7</sub>CCGCTCAGCCCCTCCTACCCACGAA 3'

2° PCR reaction mixture used:

30 50 ng DNA  
 0.5 Unit Taq polymerase (GIBCO BRL)  
 1.5 mM MgCl<sub>2</sub>  
 200 mM dNTP

0.2  $\mu$ M each primer

Total reaction volume: 25  $\mu$ l

3° PCR conditions used (on OMNIGENE HYBAID thermocycler):

- 5                    1x (5min 95°C)  
                     35x (45sec 57°C, 45sec 72°C, 45sec 95°C)  
                     1x (45sec 57°C, 15min 72°C)

4° Restriction enzyme digestion performed at 37°C for 2 hours:

- 10                   10  $\mu$ l PCR product  
                     1x BsrBI BIOLABS buffer  
                     5U BsrBI restriction enzyme (BIOLABS)  
                     Total reaction volume: 15  $\mu$ l

5° Size of fragments produced after PCR using primers with control tail and digestion with BsrBI:

- 15                   Uncut fragment from *RN* or *rn*<sup>+</sup> allele : 154 bp  
                     After digestion of fragment amplified from *RN*  
                     allele : 137 bp + 17 bp  
                     After digestion of fragment amplified from *rn*<sup>+</sup>  
                     allele : 83 bp + 54 bp + 17 bp  
                     Size difference can be identified either after  
                     polyacrylamide, agarose/NUSIEVE or agarose gel  
                     electrophoresis.

**EXAMPLE 5: EFFECT OF V40I POLYMORPHISM ON GLYCOLYTIC POTENTIAL.**

- 25                   Further, a set of 181 *rn*<sup>+</sup>/*rn*<sup>+</sup> homozygous  
                     animals (R/R at position 41 of SEQ ID NO: 2) were  
                     analyzed for the V40I polymorphism (referring to position  
                     40 of SEQ ID NO: 2) by PCR-RFLP using *Fok*I restriction  
                     enzyme. The glycolytic potential was determined in  
                     parallel according to the method disclosed by MONIN et  
                     al., (Meat Science, 13, 49-63, 1985).

The results are shown in Table 6 below:

Table 6

| Genotype at position<br>40 | Average glycolytic<br>potential | Standard Deviation | Number of typed<br>animals |
|----------------------------|---------------------------------|--------------------|----------------------------|
| I/I                        | 178.30                          | 31.13              | 13                         |
| V/I                        | 204.15                          | 37.73              | 164                        |
| V/V                        | 210.83                          | 38.21              | 104                        |

These results show that the V40I polymorphism has a significant effect on the glycolytic potential in skeletal muscle.

## CLAIMS

- 1) A gamma subunit of a vertebrate AMP-activated kinase (AMPK), wherein said gamma subunit is a polypeptide comprising at least a sequence having at  
5 least 70% identity with the polypeptide SEQ ID NO: 2.
- 2) A polypeptide of claim 1, wherein said polypeptide comprises a sequence having at least 95% identity with the polypeptide SEQ ID NO:2.
- 3) A polypeptide of claim 1, wherein said  
10 polypeptide comprises a sequence having at least 75% identity with the polypeptide SEQ ID NO: 28.
- 4) A polypeptide of any of claims 1 to 3, wherein said polypeptide comprises the sequence SEQ ID NO: 2 or SEQ ID NO:4.
- 15 5) A polypeptide of claim 4, wherein said polypeptide comprises the sequence SEQ ID NO: 28, SEQ ID NO: 30 or SEQ ID NO: 32.
- 6) A polypeptide which is a functionally altered mutant of a gamma subunit of a vertebrate AMP-  
20 activated kinase, wherein said polypeptide has at least a mutation located within the first CBS domain of said gamma subunit.
- 7) A polypeptide of claim 6, wherein the mutation is located within the region of the first CBS  
25 domain aligned with the region of a polypeptide of SEQ ID NO: 2 spanning from residue 30 to residue 50.
- 8) A polypeptide of claim 7, wherein the mutation is a R→Q substitution or a V→I substitution.
- 9) A polypeptide of claim 8 selected among:  
30 - a polypeptide having a sequence resulting from a R→Q substitution at a position corresponding to position 41 in SEQ ID NO: 2;  
- a polypeptide having a sequence resulting from a V→I substitution at the position corresponding to  
35 position 40 of SEQ ID NO: 2.

10) A polypeptide which is a mutant of a gamma subunit of a vertebrate AMP-activated kinase, wherein said polypeptide results from a deletion of a part of a polypeptide of any of claims 1 to 5.

5 11) A nucleic acid sequence encoding a polypeptide of any of claims 1 to 10, or the complement thereof, provided that said nucleic acid sequence does not consist of the EST GENBANK AA178898, or of the EST W94830.

10 12) A nucleic acid sequence of claim 11, having the sequence SEQ ID NO: 1, SEQ ID NO:3, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, or the complement thereof.

15 13) A nucleic acid sequence comprising at least a portion of a nucleic acid sequence encoding a polypeptide of any of claims 1 to 10, and up to 500 kb of a 3' and/or of a 5' adjacent genomic DNA sequence, or the complement thereof.

20 14) A nucleic acid fragment selected among:  
- a specific fragment of a nucleic acid sequence encoding a polypeptide of any of claims 1 to 10, or of a nucleic acid sequence of claim 13;  
- a nucleic acid fragment which specifically hybridises under stringent conditions with a nucleic acid sequence  
25 encoding a polypeptide of any of claims 1 to 8, or of a nucleic acid sequence of claim 11;  
provided that said nucleic acid fragment does not consist of the EST GENBANK AA178898 or of the EST GENBANK W94830.

30 15) A set of primers for amplifying a nucleic acid sequence of any of claims 11 to 13 or a portion thereof, comprising at least a primer consisting of a nucleic acid fragment of claim 14.

35 16) A recombinant vector comprising a nucleic acid sequence encoding a polypeptide of any of claims 1 to 10.

17) An host cell transformed by a nucleic acid sequence encoding a polypeptide of any of claims 1 to 10.

18) A transgenic animal transformed by a nucleic acid sequence encoding a polypeptide of any of  
5 claims 1 to 10.

19) A knockout animal, wherein the gene encoding a polypeptide of any of claims 1 to 5 is inactive.

20) A heterotrimeric AMPK wherein the  $\gamma$  subunit consists of a polypeptide of any of claims 1 to  
10 10.

21) A method of detecting a metabolic disorder resulting from a mutation in a gene encoding a  $\gamma$  subunit of AMPK, wherein said process comprises:

15 - obtaining a nucleic acid sample from a vertebrate;

- checking the presence in said nucleic acid of a nucleic acid sequence encoding a polypeptide of any of claims 1 to 10, wherein said polypeptide is  
20 functionally altered.

22) A method of claim 21 wherein the disorder is correlated with an altered glycogen accumulation in the muscular cells and results from the expression of a functionally altered allele of a polypeptide of any of  
25 claims 1 to 5.

23) A method of any of claims 21 or 22 wherein the presence of the nucleic acid sequence encoding said mutant polypeptide is checked by contacting said nucleic acid sample with a nucleic acid probe obtained from a  
30 nucleic acid of claim 14 and spanning said mutation, under conditions of specific hybridisation between said probe and the mutant sequence to be detected, and detecting the hybridisation complex.

24) A method for obtaining a pair of primers  
35 allowing to detect a genetic polymorphic marker linked to



a nucleic acid sequence encoding a polypeptide of any of claims 1 to 5, wherein said process comprises:

- screening a genomic DNA library from a vertebrate with a probe specific for a nucleic acid sequence encoding a polypeptide of any of claims 1 to 5, in order to select clones comprising said nucleic acid sequence and flanking chromosomal sequences;
- identifying a polymorphic locus in said flanking chromosomal sequences, and sequencing a DNA segment comprising said polymorphic locus ;
- designing primer pairs flanking said polymorphic locus.

25) A method of claim 24 wherein the selected clones comprise at least a portion of a nucleic acid sequence encoding a polypeptide of any of claims 1 to 5, and up to 500 kb of a 3' and/or of a 5' adjacent sequence.

26) A method of any of claims 21 to 25 wherein the vertebrate is a mammal.

27) A method of claim 26 wherein said mammal is a pig.

28) A pair of primers obtainable by the process of any of claims 24 to 26.

29) A process for detecting a dysfunction of carbohydrate metabolism resulting from the expression of a functionally altered allele of a polypeptide of any of claims 1 to 5 in a vertebrate, wherein said process comprises:

- obtaining a sample of genomic DNA from said vertebrate;
- contacting said DNA with a pair of primers of claim 28 under conditions allowing PCR amplification;
- analysing the PCR product to detect if an allele of a polymorphic marker linked to a nucleic acid sequence encoding a functionally altered allele of a polypeptide of any of claims 1 to 5 is present.

30) A process of claim 29, wherein said functionally altered polypeptide results from a R41Q substitution in SEQ ID NO: 2.

31) A process of any of claims 29 or 30, wherein said vertebrate is a mammal.

32) A process of claim 31 wherein said mammal is a pig.

33) A process of claim 32 wherein the pair of primers is selected among:

- a pair of primers consisting of SEQ ID NO: 5 and SEQ ID NO: 6;

- a pair of primers consisting of SEQ ID NO: 7 and SEQ ID NO: 8;

- a pair of primers consisting of SEQ ID NO: 9 and SEQ ID NO: 10;

- a pair of primers consisting of SEQ ID NO: 11 and SEQ ID NO: 12;

- a pair of primers consisting of SEQ ID NO: 13 and SEQ ID NO: 14;

- a pair of primers consisting of SEQ ID NO: 15 and SEQ ID NO: 16;

- a pair of primers consisting of SEQ ID NO: 17 and SEQ ID NO: 18;

- a pair of primers consisting of SEQ ID NO: 19 and SEQ ID NO: 20;

- a pair of primers consisting of SEQ ID NO: 21 and SEQ ID NO: 22;

- a pair of primers consisting of SEQ ID NO: 23 and SEQ ID NO: 24;

- a pair of primers consisting of SEQ ID NO: 25 and SEQ ID NO: 26.

34) Use of a transformed cell of claim 17 to screen compounds able to modulate AMPK activity.

35) Use of a transgenic animal of claim 18 to screen compounds able to modulate AMPK activity.

36) Use of a knockout animal of claim 19 to screen compounds able to modulate energy metabolism in the absence of a functional polypeptide of any of claims 1 to 5.

5           37) Use of an heterotrimeric AMPK of claim 20 to screen compounds able to modulate AMPK activity.

1/8

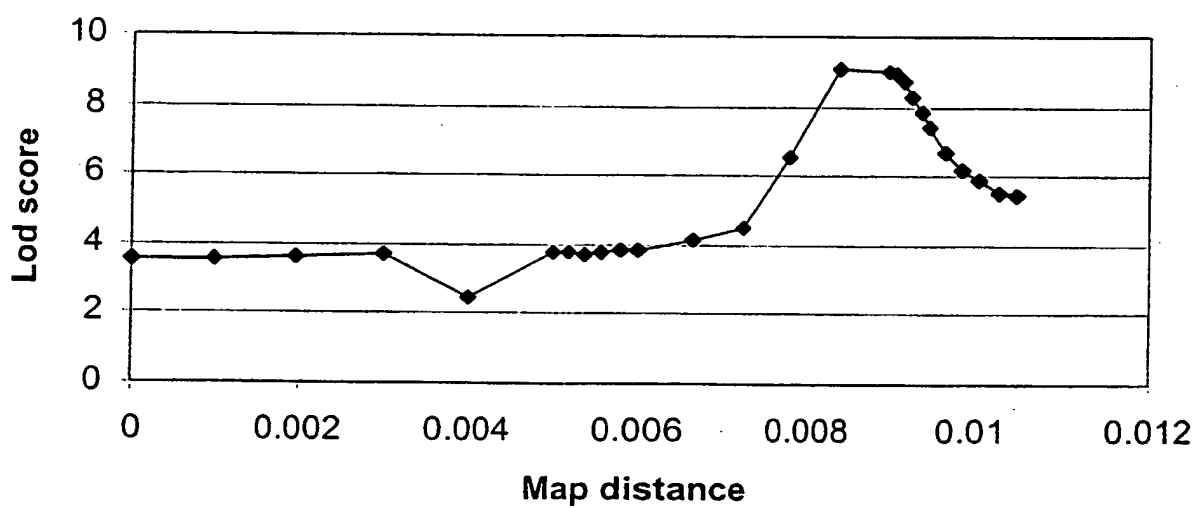


Figure 1

|     |  |     |     |     |     |     |     |       |     |
|-----|--|-----|-----|-----|-----|-----|-----|-------|-----|
|     | 5'UTR  | 10  | 20  | 30  | 40  | 50  | 60  | 70    | 80  |
| Pig | TTCCTAGAGCAAGGAGAGAGCCGTTTCATGGCCATCCCGAGCTGTAACCACCAGCTCAGAAAGAAGCCATGGGGACCAGGG  |     |     |     |     |     |     |       |     |
| Hum | -----A-A-C---A-C-----A-C-----G---G-----T--G---A-A-G-A-                             |     |     |     |     |     |     |       |     |
|     | 90   | 100 | 110 | 120 | 130 | 140 | 150 | 160   |     |
| Pig | GAACAAGGCCTCTAGATGGACAAGGCAGGAGGATGTAGAGGAAGGGGGGCTCCGGGCCCCGAGGGAAGGTCCCCAGTCCA   |     |     |     |     |     |     |       |     |
| Hum | -GC---A---TG-----A---TCG---G---A---A---A---T-A-G-----G-----                        |     |     |     |     |     |     |       |     |
|     | 170  | 180 | 190 | 200 | 210 | 220 | 230 | 240   |     |
| Pig | GGCCAGTTGCTGAGTCCACCGGGCAGGAGGCCACATTCCTCCCAAGGCCACACCCTTGGCCCAAGCCGCTCCCTTGGCCGAG |     |     |     |     |     |     |       |     |
| Hum | -----AC-----T-----A-----T-----T-A-----T-----G---                                   |     |     |     |     |     |     |       |     |
|     | 250  | 260 | 270 | 280 | 290 | 300 | 310 | 320   |     |
| Pig | GTGGACAACCCCCAACAGAGCGGGACATCCTCCCTCTGACTGTGCAGCCTCAGCCTCCGACTCCAACACAGACCATCT     |     |     |     |     |     |     |       |     |
| Hum | -----G---CT--A-----G-T---TG-----A-----TG-A-G-----G---TG--G-                        |     |     |     |     |     |     |       |     |
|     | 330  | 340 | 350 | 360 | 370 | 380 | 390 | 400   |     |
| Pig | GGATCTGGGCATAGAGTTCTCAGCCTCGGCGGCGTGGGGGATGAGCTTG...GGCTGGTGAAGAGAAGCCAGCCCCGT     |     |     |     |     |     |     |       |     |
| Hum | ---G---C---CG-----C-----A-A-A---C-G---A-TG-----A-AAG-C---C-----G---T---T---        |     |     |     |     |     |     |       |     |
|     | 410  | 420 | 430 | 440 | 450 | 460 | 470 | 5'UTR |     |
| Pig | GCCCATCCCCAGAGGTGCTGTTACCCAGGCTGGGCTGGGATGATGAGCTGCAGAAGCCGGGGGCCAGGTCTAC          |     |     |     |     |     |     |       |     |
| Hum | ---TG---GC---CC-CA--T---A-----C--A---G---A--C--C-----A-----                        |     |     |     |     |     |     |       |     |
|     | CDS  |     |     | 10  |     |     |     |       | 20  |
| Pig | ATG CAC TTC ATG CAG GAG CAC ACC TGC TAC GAT GCC ATG GCG ACC AGC TCC AAA CTG GTC    |     |     |     |     |     |     |       |     |
| Met | His Phe Met Gln Glu His Thr Cys Tyr Asp Ala Met Ala Thr Ser Ser Lys Leu Val        |     |     |     |     |     |     |       |     |
| Hum | ---G--- --- --- --- --- --- --- --- --- --- ---A---T--- --- ---G---A---            |     |     |     |     |     |     |       |     |
|     | Arg  |     |     |     |     |     |     |       |     |
|     |  |     |     | 30  |     |     |     |       | 40  |
| Pig | ATC TTC GAC ACC ATG CTG GAG ATC AAG AAG GCC TTC TTT GCC CTG GTG GCC AAC GGC GTC    |     |     |     |     |     |     |       |     |
| Ile | Phe Asp Thr Met Leu Glu Ile Lys Lys Ala Phe Phe Ala Leu Val Ala Asn Gly Val        |     |     |     |     |     |     |       |     |
| Hum | --- --- --- --- --- --- --- --- --- ---T--- --- --- ---T---G---                    |     |     |     |     |     |     |       |     |
|     |  |     |     |     |     |     |     |       |     |
|     |  |     |     | 50  |     |     |     |       | 60  |
| Pig | CGA GCG GCA CCT TTG TGG GAC AGC AAG AAG CAG AGC TTC GTG GGG ATG CTG ACC ATC ACA    |     |     |     |     |     |     |       |     |
| Arg | Ala Ala Pro Leu Trp Asp Ser Lys Lys Gln Ser Phe Val Gly Met Leu Thr Ile Thr        |     |     |     |     |     |     |       |     |
| Hum | --G--A--C---C-A--- --- --- --- --- ---T--- --- --- --- ---T---                     |     |     |     |     |     |     |       |     |
|     |  |     |     |     |     |     |     |       |     |
|     |  |     |     | 70  |     |     |     |       | 80  |
| Pig | GAC TTC ATC TTG GTG CTG CAC CGC TAT TAC AGG TCC CCC CTG GTC CAG ATC TAC GAG ATT    |     |     |     |     |     |     |       |     |
| Asp | Phe Ile Leu Val Leu His Arg Tyr Tyr Arg Ser Pro Leu Val Gln Ile Tyr Glu Ile        |     |     |     |     |     |     |       |     |
| Hum | --- --- --- C--- --- ---T--- ---C--- --- --- --- ---T--- --- ---                   |     |     |     |     |     |     |       |     |
|     |  |     |     |     |     |     |     |       |     |
|     |  |     |     | 90  |     |     |     |       | 100 |
| Pig | GAA GAA CAT AAG ATT GAG ACC TGG AGG GAG ATC TAC CTT CAA GGC TGC TTC AAG CCT CTG    |     |     |     |     |     |     |       |     |
| Glu | Glu His Lys Ile Glu Thr Trp Arg Glu Ile Tyr Leu Gln Gly Cys Phe Lys Pro Leu        |     |     |     |     |     |     |       |     |
| Hum | --- C--- --- --- --- --- --- --- ---G--- --- --- --- --- ---                       |     |     |     |     |     |     |       |     |
|     | Gln  |     |     |     |     |     |     |       |     |
|     |  |     |     | 110 |     |     |     |       | 120 |
| Pig | GTC TCC ATC TCT CCC AAT GAC AGC CTG TTC GAA GCT GTC TAC GCC CTC ATC AAG AAC CGG    |     |     |     |     |     |     |       |     |
| Val | Ser Ile Ser Pro Asn Asp Ser Leu Phe Glu Ala Val Tyr Ala Leu Ile Lys Asn Arg        |     |     |     |     |     |     |       |     |
| Hum | --- --- --- ---T--- ---T--- ---T--- --- --- ---A--- --- --- --- ---                |     |     |     |     |     |     |       |     |
|     |  |     |     |     |     |     |     | Thr   |     |
|     |  |     |     | 130 |     |     |     |       | 140 |
| Pig | ATC CAC CGC CTG CCG GTC CTG GAC CCT GTC TCC GGG GCT GTG CTC CAC ATC CTC ACA CAT    |     |     |     |     |     |     |       |     |
| Ile | His Arg Leu Pro Val Leu Asp Pro Val Ser Gly Ala Val Leu His Ile Leu Thr His        |     |     |     |     |     |     |       |     |
| Hum | ---T--- --- ---T---T---T--- ---G---G---A---C---AAC---A--- --- --- ---C---          |     |     |     |     |     |     |       |     |
|     |  |     |     |     |     |     |     | Asn   |     |
|     |  |     |     | 150 |     |     |     |       | 160 |
| Pig | AAG CGG CTT CTC AAG TTC CTG CAC ATC TTT GGC ACC CTG CTG CCC CGG CCC TCC TTC CTC    |     |     |     |     |     |     |       |     |
| Lys | Arg Leu Leu Lys Phe Leu His Ile Phe Gly Thr Leu Leu Pro Arg Pro Ser Phe Leu        |     |     |     |     |     |     |       |     |
| Hum | --A--C--G--- --- --- --- ---T---T--- --- --- --- --- --- --- ---                   |     |     |     |     |     |     |       |     |
|     |  |     |     |     |     |     |     | Ser   |     |

Figure 2

|     | 190 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 200 |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pig | ACG | GCG | CCC | ATC | CTG | ACC | GCA | CTG | GAC | ATC | TTC | GTG | GAC | CGG | CGT | GTG | TCT | GCG | CTG | CCT |
|     | Thr | Ala | Pro | Ile | Leu | Thr | Ala | Leu | Asp | Ile | Phe | Val | Asp | Arg | Arg | Val | Ser | Ala | Leu | Pro |
| Hum | --A | --A | --- | --- | --- | --T | --- | --- | --- | --- | --T | --- | --- | --- | --- | --- | --- | --A | --- | --- |

[illegible]

|     | 230 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 240  |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| Pig | GCT | GCC | CAA | CAA | ACA | TAC | AAC | CAC | CTG | GAC | ATG | AAT | GTG | GGA | GAA | GCC | CTG | AGG | CAG | CGG  |
|     | Ala | Ala | Gln | Gln | Thr | Tyr | Asn | His | Leu | Asp | Met | Asn | Val | Gly | Glu | Ala | Leu | Arg | Gln | Arg  |
| Hum | --- | --- | --G | --- | --C | --- | --- | --- | --- | --- | --- | -G- | --- | --- | --- | --- | --- | --- | --- | A--- |
|     | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | Ser | -   | -   | -   | -   | -   | -   | -   | -    |

|     | 250 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 260 |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pig | ACA | CTG | TGT | CTG | GAA | GGC | GTC | CTT | TCC | TGC | CAG | CCC | CAC | GAG | ACC | TTG | GGG | GAA | GTC | ATT |     |
|     | Thr | Leu | Cys | Leu | Glu | Gly | Val | Leu | Ser | Cys | Gln | Pro | His | Glu | Thr | Leu | Gly | Glu | Val | Ile |     |
| Hum | --- | --A | --- | --- | --G | --A | --- | --- | --- | --- | --- | --- | --- | --- | -G- | --- | --- | --- | --G | --C |     |

[illegible]

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|     |     |     |     |     |     |     |     |     | 290 |     |     |     |     |     |     |     |     |     | 300 |     |
| Pig | CTG | GGC | GTG | GTG | TCC | CTC | TCT | GAC | ATC | CTT | CAG | GCT | CTG | GTG | CTC | AGC | CCT | GCT | GGA | ATT |
|     | Leu | Gly | Val | Val | Ser | Leu | Ser | Asp | Ile | Leu | Gln | Ala | Leu | Val | Leu | Ser | Pro | Ala | Gly | Ile |
| Hum | T-- | --- | --- | --C | --- | --- | --C | --- | --- | --- | --- | --A | --- | --- | --- | --- | --- | --- | --C | --C |

|     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|
|     |     |     |     |     |     | CDS |
| Pig | GAT | GCC | CTC | GGG | GCC | TGA |
|     | Asp | Ala | Leu | Gly | Ala | *** |
| Hum | --- | --- | --- | --- | --- | --- |

|     | 3'UTR   | 10  | 20  | 30  | 40  | 50  | 60  | 70  | 80  |
|-----|---|-----|-----|-----|-----|-----|-----|-----|-----|
| Pig | GAACCTTGGAACCTTTGCTCTCAGGCCACCTGGCACACCTTGAAGCCAGTGAAGGGAGCCGTGGACTCAGCTCTCACTTC  |     |     |     |     |     |     |     |     |
| Hum | ---GA-CT--GT-C-CAA--C-A-----A-----A-----A-----A-T....AGAA-----T---                |     |     |     |     |     |     |     |     |
|     |   | 90  | 100 | 110 | 120 | 130 | 140 | 150 | 160 |
| Pig | CCCTCAGCCCCACTTGCTGGTCTGGCTCTTGTTTCAGGTAGGCTCCGCCCGGGGC...CCCTGGCCTCAGCATCAGCCC   |     |     |     |     |     |     |     |     |
| Hum | ---A.-C---A-T-----TCA---A-GA-----CTTCT--A---TTCCAAAATTG--T-T---T-.T-GT--T-        |     |     |     |     |     |     |     |     |
|     |   | 170 | 180 | 190 | 200 | 210 | 220 | 230 | 240 |
| Pig | CTCAGTCTCCCT.GGGCACCCAGATCTCAGACTGGGGCACCCCTGAAGATG.GGAGTGGCCAGCTTATAGCTGAGCAG.C  |     |     |     |     |     |     |     |     |
| Hum | ----AAC--T-C-----TG-CC-GTG--CCA-----TGA---AT-AA---AACAG-T-AG-CA----TG-AG-T-       |     |     |     |     |     |     |     |     |
|     |   | 250 | 260 | 270 | 280 | 290 | 300 | 310 | 320 |
| Pig | CTTGTG..AAATCTACCAGCATCAAGACT...CACTGTGGGACCACTGCTTTG...TCCCATTCTCAGCTGAAATGAT.G  |     |     |     |     |     |     |     |     |
| Hum | -C--AACC-G-GGC--T--G--T-CCC-AGGG-CA-C--T-CT-CA---CCGCCCA----C--GC-GC-----CTG-G-C- |     |     |     |     |     |     |     |     |
|     |   | 330 | 340 | 350 | 360 | 370 | 380 | 390 | 400 |
| Pig | GAGGGCCTCATAAGAGGGGTGGACAGGGC..CTGGAGTAGAGGCCAGATCAGTGACGT..GCCTTCAGG...ACCTCCG   |     |     |     |     |     |     |     |     |
| Hum | --T---C--GTG---TT-A-T-----TT-----T-CCTC-GTTTC-GG-CT--C-AT-G-----CCTTC-G----       |     |     |     |     |     |     |     |     |

RECTIFIED SHEET (RULE 91) ISA/EP

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|     |   |     |     |     |     |     |     |     |
|-----|---|-----|-----|-----|-----|-----|-----|-----|
|     | 410   | 420 | 430 | 440 | 450 | 460 | 470 | 480 |
| Pig | GGGAGTTAGAGCTGCCCTCTCTCAGTT.....CAGTTCCCCCTGCTGAGAATG.TCCCTGGAAGGAAGCCAGTTAAT   |     |     |     |     |     |     |     |
| Hum | -----CCC-----TTG---C---CAACGTGGC--C-G---T---A--CTCC-G-C-TTG-CATTTC---G-T-C-G--  |     |     |     |     |     |     |     |
|     | 490   | 500 | 510 | 520 | 530 | 540 | 550 | 560 |
| Pig | AAACCTTGGTTGGATGGAATTTCCACACTCG.....  |     |     |     |     |     |     |     |
| Hum | --TG--GCA--TC-G--G.....CA-G-AGCAGCCGTTATTTATAGAACTGCCTGTTGGAGGTGGGGAGTCCTCCCT   |     |     |     |     |     |     |     |
|     | 570   | 580 | 590 | 600 | 610 | 620 | 630 | 640 |
| Pig | .....   |     |     |     |     |     |     |     |
| Hum | CCATTCTTGTCAGAAAACTCCTTAGCTCTCGCAGTGAGCCATGTTCTTAGTCTCCAGGGATGGATGGCCTTGTATATGG |     |     |     |     |     |     |     |
|     | 650   | 660 | 670 | 680 | 690 | 700 | 710 | 720 |
| Pig | .....   |     |     |     |     |     |     |     |
| Hum | ACCCCTGAGAATGAGCAATTGAGAAAACAAAACAAAAGGAACAATCCATGAACTTAGATTTATTGGTTTCACTCAAAAT |     |     |     |     |     |     |     |
|     | 730   | 740 |     |     |     |     |     |     |
| Pig | .....   |     |     |     |     |     |     |     |
| Hum | GCTGCAGTCATTTGACCTG   |     |     |     |     |     |     |     |

Figure 2 (cont.)

PigG3 MSFLEQGESRSPSRAVTTSSERSHGDQCNKASRWTRQEDVEEGPPGREGPQSRPVAESTGQATFPKATPLAQAPLAENVDPPTERDILPSCDAAS 100  
HumG3 -----EN-S-----P-----S-----IR-KRRA--L-----KS-----E-----QG-----R-----T-----L-----T-----D-----G-GT-----GW-C-----T----- 200  
CB81→

PigG3 ASDSNTDHLDLGIEFSASAASGDEL.GLVEEKPAPCPSPPEVLLPRLGWDELQKPGAQVYMIQMHEHTCYDAMATSSKLVIPTMTLEIKKAFFALVANGV  
HumG3 -AG-S--DVE-AT--P-TE-WEC--E--L--R--L-L--QAPP-K-  
HumG1 METVIS-DSSPAVENEHPQ-TPESNNS--TS--KS-R--LIP-----V--S-QV-----T-----  
HumG2 AALGPAEAGM-EKLEFE-EAVEDSESG--R--RS-K--IVP-----V--T-QV-----T-----  
Drob RDSRGLPVADPPFLEKVNLS-DEEDS-IFVK-FRP-K--LIP-A--V--Q-LV-----Y-----Y-----  
Snf4 MK-TQDSQEKVSIEQQLAVES..IRK-LNSK-S--VLPV-YR-IVL--S-LV--SLNV-LQ-SI  
CB82→ 300

PigG3 RAAPLWDSKKQSFVGMCTITDFILVLRHYRSPLQIYEIEENKIETWREIYLGCFKPL..VSTSPNDSLFEAVALIKNRIHRLPVLDPVSGA...V  
HumG3 -----NI-----K-A-----L-----V--DS-----C--A--D--SS--R-K-----I--E--N-----T-----  
HumG1 -----E--T--SL-----NI-----K-M-----L-----L--ET-----N--DA--D--S--K-----I--I--N-----A-----  
Drob -----E--Q-----KI-QM--K--NASMEQL--LD--DV--HNQVM-----G-DA--YD-IKI--HS-----I--AT--N-----  
Snf4 VS-----TSR-A-L--T-----N-IQY-FSN-D.KFELVDKLQDLGLKD-ERALGVDQ-DTA--H-SRP-----CLKMLES-SG-I-LI-QDEETHREI-  
CB83→ 400

PigG3 LHILTHKRLLKFLHIFGTLPRPSPFLYRTIQDLGIGTFRDLAVVLETPALITALDIFVDRRVSALPVVNETGQWGLYSRFDVIHLAAQQTYNHILDMNVG  
HumG3 -----S-----S-----A-----C-----S-----  
HumG1 -Y-----I-----KL-I-EF-K-E-MKSLEE-Q--YANI-M-RT-T-VYV--G--QH-----D-K-R--DI--K--N--EK--N--VS-T  
HumG2 -Y-----I-----QL-MSDM-K-A-MKQNLDE-----YHNI-FIHPT--IK-N--E--I-----D-S-K--DI--K--N--EK--N--IT-T  
Drob -Y-----I-R--FLYINE--K-AYMQKSLRE-K--YNNIETAD--TS-I--KK--E-----L-DSD-RL-DI-AK--N--EK--D--VSLR  
Snf4 VSV--QY-I-----VALNCRE...TH--KIP-G--N-I-QDNMKSCQM-T-VIDVIQMLTQG--SV-IID-N-YLINV-EAY--LG-IKGI--D-SLS--  
CB84→ 472

PigG3 EALRQRTLCLGVLSQCPHETLGEVIDRIVREQVHRLVLVDETQHLGLGVVLSLSDILQALVLSPAGIDALGA\*  
HumG3 -----S-----S-----A-----A-----  
HumG1 K--QH-SHYF--K-YL-----ETI-N-L-EAE-----V--NDVVK-I-----TGGEKKP\*  
HumG2 Q--QH-SQYF--VK-NKL-I-ETIV-----AE-----V-N-ADSIV-II-----I-T--AKQKETETE\*  
Drob K-NEH-NEWF--QK-NLD-S-YTIME-----AE-----V--NRKVI-II-----LY--R-S-EGV  
Snf4 ---MR-SDDF--YT-TKNDK-STIM-N-RKAR--FFV--DVGR-V--LT-----KYIL-GSN\*

Figure 3



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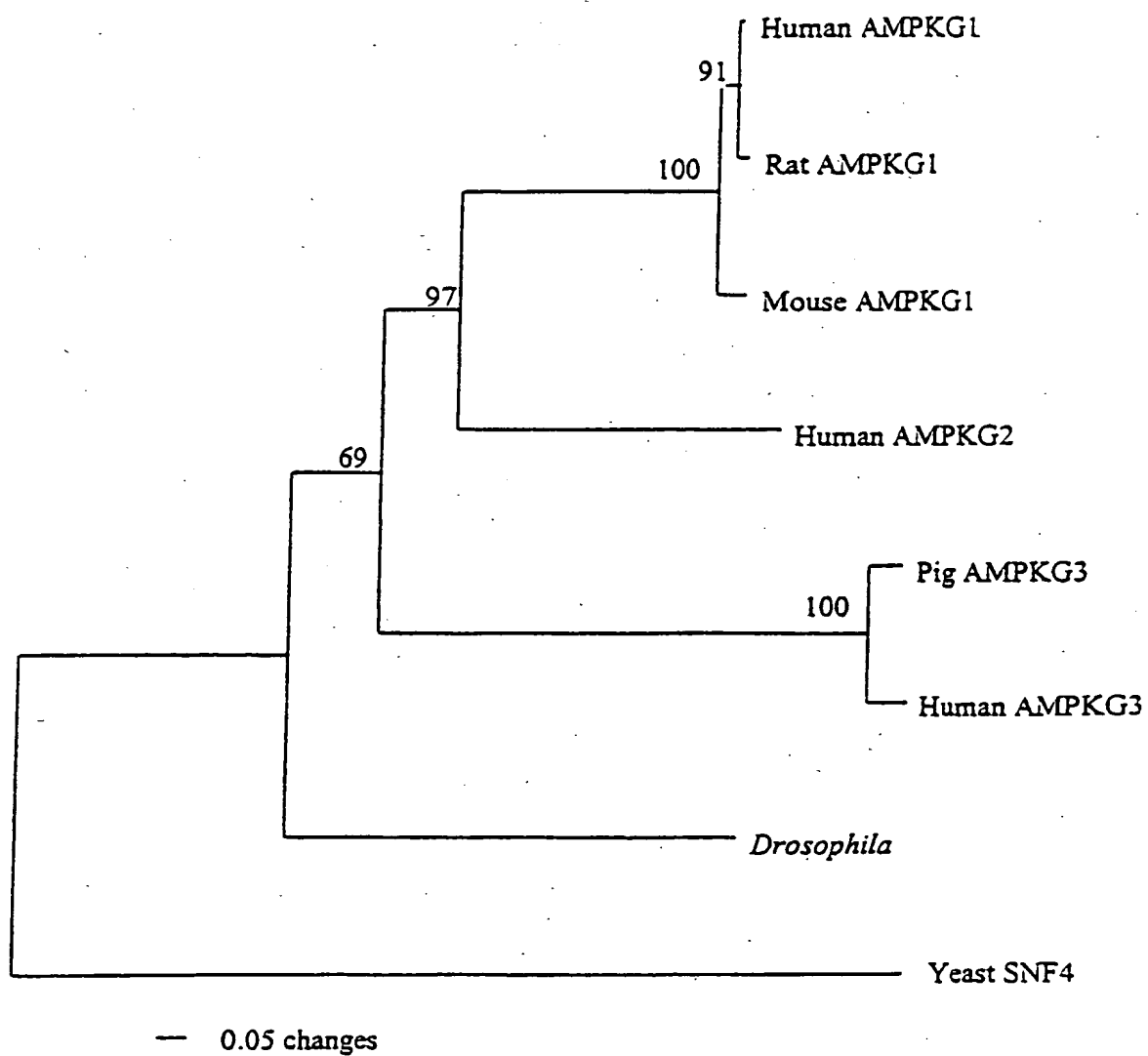


Figure 4

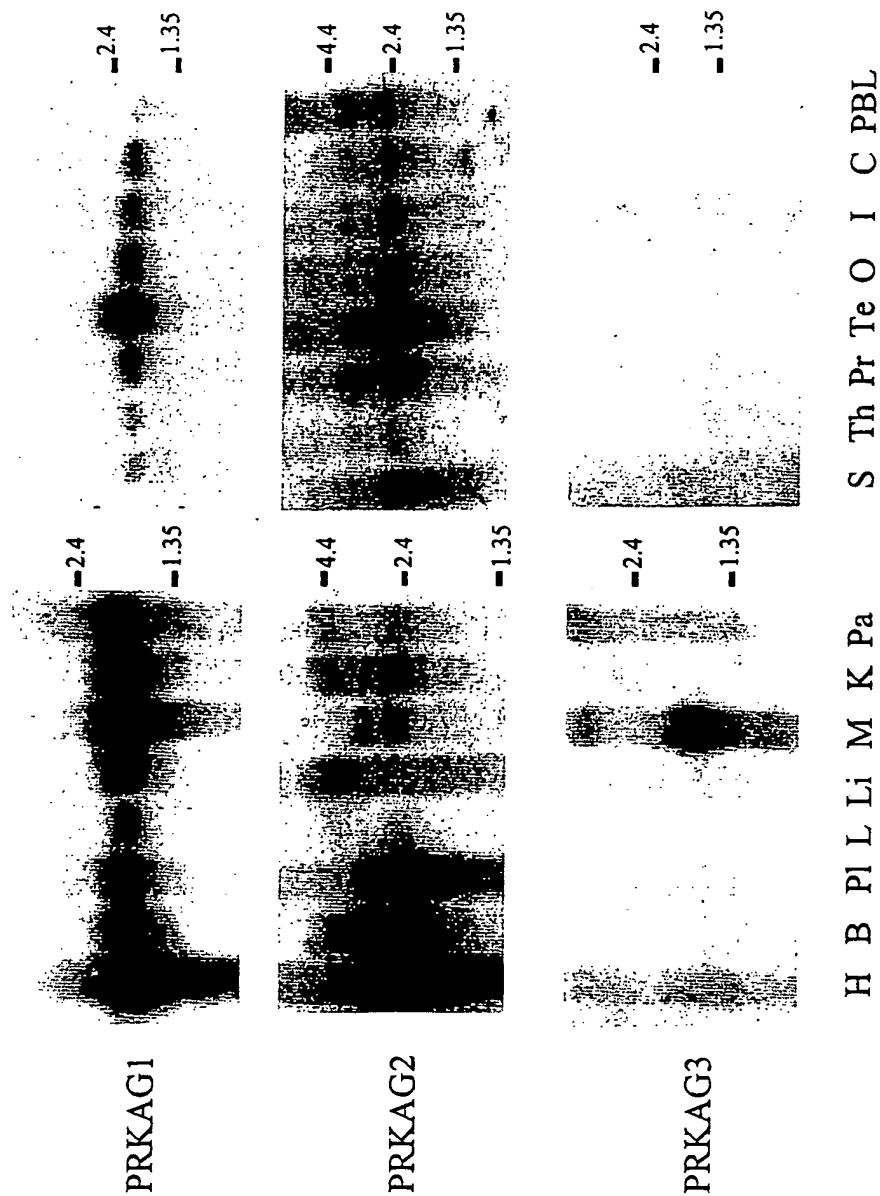


Figure 5

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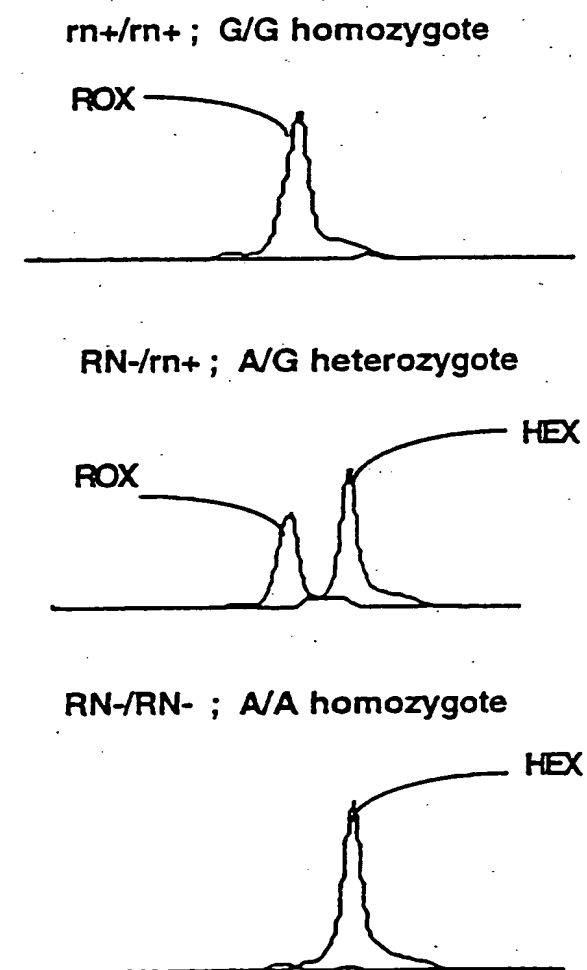


Figure 6

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## SEQUENCE LISTING

&lt;110&gt; INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE

MILAN, Denis

ANDERSSON, Leif

LOOFT, Christian

ROBIC, Annie

ROGEL-GAILLARD, Claire

IANNUCCELLI, Nathalie

GELLIN, Joël

KALM, Ernst

LE ROY, Pascale

CHARDON, Patrick

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| 20 25 30  |      |
| ctg gtg gcc aac ggc gtc cga gcg gca cct ttg tgg gac agc aag aag | 621  |
| Leu Val Ala Asn Gly Val Arg Ala Ala Pro Leu Trp Asp Ser Lys Lys |      |
| 35 40 45 50   |      |
| cag agc ttc gtg ggg atg ctg acc atc aca gac ttc atc ttg gtg ctg | 669  |
| Gln Ser Phe Val Gly Met Leu Thr Ile Thr Asp Phe Ile Leu Val Leu |      |
| 55 60 65  |      |
| cac cgc tat tac agg tcc ccc ctg gtc cag atc tac gag att gaa gaa | 717  |
| His Arg Tyr Tyr Arg Ser Pro Leu Val Gln Ile Tyr Glu Ile Glu Glu |      |
| 70 75 80  |      |
| cat aag att gag acc tgg agg gag atc tac ctt caa ggc tgc ttc aag | 765  |
| His Lys Ile Glu Thr Trp Arg Glu Ile Tyr Leu Gln Gly Cys Phe Lys |      |
| 85 90 95  |      |
| cct ctg gtc tcc atc tct ccc aat gac agc ctg ttc gaa gct gtc tac | 813  |
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| gcc ctc atc aag aac cgg atc cac cgc ctg ccg gtc ctg gac cct gtc | 861  |
| Ala Leu Ile Lys Asn Arg Ile His Arg Leu Pro Val Leu Asp Pro Val |      |
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| Ser Gly Ala Val Leu His Ile Leu Thr His Lys Arg Leu Leu Lys Phe |      |
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| ctg gaa acg gcg ccc atc ctg acc gca ctg gac atc ttc gtg gac cgg | 1053 |
| Leu Glu Thr Ala Pro Ile Leu Thr Ala Leu Asp Ile Phe Val Asp Arg |      |
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| 215 220 225   |      |
| aac cac ctg gac atg aat gtg gga gaa gcc ctg agg cag cgg aca ctg | 1197 |
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| Cys Leu Glu Gly Val Leu Ser Cys Gln Pro His Glu Thr Leu Gly Glu |      |
| 245 250 255   |      |

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Tyr Arg Thr Ile Gln Asp Leu Gly Ile Gly Thr Phe Arg Asp Leu Ala  
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Val Val Leu Glu Thr Ala Pro Ile Leu Thr Ala Leu Asp Ile Phe Val  
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Asp Arg Arg Val Ser Ala Leu Pro Val Val Asn Glu Thr Gly Gln Val  
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Val Gly Leu Tyr Ser Arg Phe Asp Val Ile His Leu Ala Ala Gln Gln  
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Thr Tyr Asn His Leu Asp Met Asn Val Gly Glu Ala Leu Arg Gln Arg  
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Gly Glu Val Ile Asp Arg Ile Val Arg Glu Gln Val His Arg Leu Val  
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Leu Val Asp Glu Thr Gln His Leu Leu Gly Val Val Ser Leu Ser Asp  
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Ala  
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|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |         |  |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|---------|--|
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | Met Arg |  |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 1       |  |
| ttc | atg | cag | gag | cac | acc | tgc | tac | gat | gcc | atg | gca | act | agc | tcc | aag | 525     |  |
| Phe | Met | Gln | Glu | His | Thr | Cys | Tyr | Asp | Ala | Met | Ala | Thr | Ser | Ser | Lys |         |  |
| 5   |     |     |     | 10  |     |     |     | 15  |     |     |     |     |     |     |     |         |  |
| cta | gtc | atc | ttc | gac | acc | atg | ctg | gag | atc | aag | aag | gcc | ttc | ttt | gct | 573     |  |
| Leu | Val | Ile | Phe | Asp | Thr | Met | Leu | Glu | Ile | Lys | Lys | Ala | Phe | Phe | Ala |         |  |
| 20  |     |     |     | 25  |     |     |     | 30  |     |     |     |     |     |     |     |         |  |
| ctg | gtg | gcc | aac | ggg | gtg | cgg | gca | gcc | cct | cta | tgg | gac | agc | aag | aag | 621     |  |
| Leu | Val | Ala | Asn | Gly | Val | Arg | Ala | Ala | Pro | Leu | Trp | Asp | Ser | Lys | Lys |         |  |
| 35  |     |     |     | 40  |     |     |     | 45  |     |     |     | 50  |     |     |     |         |  |
| cag | agc | ttt | gtg | ggg | atg | ctg | acc | atc | act | gac | ttc | atc | ctg | gtg | ctg | 669     |  |
| Gln | Ser | Phe | Val | Gly | Met | Leu | Thr | Ile | Thr | Asp | Phe | Ile | Leu | Val | Leu |         |  |
|     |     |     |     | 55  |     |     |     | 60  |     |     |     | 65  |     |     |     |         |  |
| cat | cgc | tac | tac | agg | tcc | ccc | ctg | gtc | cag | atc | tat | gag | att | gaa | caa | 717     |  |
| His | Arg | Tyr | Tyr | Arg | Ser | Pro | Leu | Val | Gln | Ile | Tyr | Glu | Ile | Glu | Gln |         |  |
| 70  |     |     |     | 75  |     |     |     | 80  |     |     |     |     |     |     |     |         |  |
| cat | aag | att | gag | acc | tgg | agg | gag | atc | tac | ctg | caa | ggc | tgc | ttc | aag | 765     |  |
| His | Lys | Ile | Glu | Thr | Trp | Arg | Glu | Ile | Tyr | Leu | Gln | Gly | Cys | Phe | Lys |         |  |
| 85  |     |     |     | 90  |     |     |     | 95  |     |     |     |     |     |     |     |         |  |
| cct | ctg | gtc | tcc | atc | tct | cct | aat | gat | agc | ctg | ttt | gaa | gct | gtc | tac | 813     |  |
| Pro | Leu | Val | Ser | Ile | Ser | Pro | Asn | Asp | Ser | Leu | Phe | Glu | Ala | Val | Tyr |         |  |
| 100 |     |     |     | 105 |     |     |     | 110 |     |     |     |     |     |     |     |         |  |
| acc | ctc | atc | aag | aac | cgg | atc | cat | cgc | ctg | cct | gtt | ctt | gac | ccg | gtg | 861     |  |
| Thr | Leu | Ile | Lys | Asn | Arg | Ile | His | Arg | Leu | Pro | Val | Leu | Asp | Pro | Val |         |  |
| 115 |     |     |     | 120 |     |     |     | 125 |     |     |     | 130 |     |     |     |         |  |
| tca | ggc | aac | gta | ctc | cac | atc | ctc | aca | cac | aaa | cgc | ctg | ctc | aag | ttc | 909     |  |
| Ser | Gly | Asn | Val | Leu | His | Ile | Leu | Thr | His | Lys | Arg | Leu | Leu | Lys | Phe |         |  |
|     |     |     |     | 135 |     |     |     | 140 |     |     |     | 145 |     |     |     |         |  |
| ctg | cac | atc | ttt | ggg | tcc | ctg | ctg | ccc | cgg | ccc | tcc | ttc | ctc | tac | cgc | 957     |  |
| Leu | His | Ile | Phe | Gly | Ser | Leu | Leu | Pro | Arg | Pro | Ser | Phe | Leu | Tyr | Arg |         |  |
| 150 |     |     |     | 155 |     |     |     | 160 |     |     |     |     |     |     |     |         |  |
| act | atc | caa | gat | ttg | ggc | atc | ggc | aca | ttc | cga | gac | ttg | gct | gtg | gtg | 1005    |  |
| Thr | Ile | Gln | Asp | Leu | Gly | Ile | Gly | Thr | Phe | Arg | Asp | Leu | Ala | Val | Val |         |  |
| 165 |     |     |     | 170 |     |     |     | 175 |     |     |     |     |     |     |     |         |  |
| ctg | gag | aca | gca | ccc | atc | ctg | act | gca | ctg | gac | atc | ttt | gtg | gac | cgg | 1053    |  |
| Leu | Glu | Thr | Ala | Pro | Ile | Leu | Thr | Ala | Leu | Asp | Ile | Phe | Val | Asp | Arg |         |  |
| 180 |     |     |     | 185 |     |     |     | 190 |     |     |     |     |     |     |     |         |  |
| cgt | gtg | tct | gca | ctg | cct | gtg | gtc | aac | gaa | tgt | ggg | cag | gtc | gtg | ggc | 1101    |  |
| Arg | Val | Ser | Ala | Leu | Pro | Val | Val | Asn | Glu | Cys | Gly | Gln | Val | Val | Gly |         |  |
| 195 |     |     |     | 200 |     |     |     | 205 |     |     |     | 210 |     |     |     |         |  |
| ctc | tat | tcc | cgc | ttt | gat | gtg | att | cac | ctg | gct | gcc | cag | caa | acc | tac | 1149    |  |
| Leu | Tyr | Ser | Arg | Phe | Asp | Val | Ile | His | Leu | Ala | Ala | Gln | Gln | Thr | Tyr |         |  |
| 215 |     |     |     | 220 |     |     |     | 225 |     |     |     |     |     |     |     |         |  |



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aac cac ctg gac atg agt gtg gga gaa gcc ctg agg cag agg aca cta 1197  
 Asn His Leu Asp Met Ser Val Gly Glu Ala Leu Arg Gln Arg Thr Leu  
                   230                                  235                                  240

tgt ctg gag gga gtc ctt tcc tgc cag ccc cac gag agc ttg ggg gaa 1245  
 Cys Leu Glu Gly Val Leu Ser Cys Gln Pro His Glu Ser Leu Gly Glu  
                   245                                  250                                  255

gtg atc gac agg att gct cgg gag cag gta cac agg ctg gtg cta gtg 1293  
 Val Ile Asp Arg Ile Ala Arg Glu Gln Val His Arg Leu Val Leu Val  
                   260                                  265                                  270

gac gag acc cag cat ctc ttg ggc gtg gtc tcc ctc tcc gac atc ctt 1341  
 Asp Glu Thr Gln His Leu Leu Gly Val Val Ser Leu Ser Asp Ile Leu  
                   275                                  280                                  285                                  290

cag gca ctg gtg ctc agc cct gct ggc atc gat gcc ctc ggg gcc tga 1389  
 Gln Ala Leu Val Leu Ser Pro Ala Gly Ile Asp Ala Leu Gly Ala  
                                   295                                  300                                  305

gaagatctga gtcctcaatc ccaagccaac tgcacactgg aagccaatga aggaattgag 1449

aacagcttca tttccccaac cccaatttgc tggttcagct atgattcagg cttcttcagc 1509

cttccaaaat tgcctttgcc ttacttgtgc tcccagaacc cttcgggcat gccagtgca 1569

ccatgggatg atgaaattaa ggagaacagc tgagtcaagc ttggaggtcc ctgaaccaga 1629

ggcactagga ttaccccagg gccatctgtg ctccatgcc gcccatcccc ttgccgcctg 1689

actgggtcgg atggccccag tgggtttagt cagggcttct ggattcctcg gtttctgggc 1749

tacctatggc ttcagccttc agtcctggg agtcccagct gttgttcca gcaacgtcgc 1809

cactgccctc ctactctcca ggctttgtca tttcaaggct gctgaaatgc tgcatttcag 1869

gggccaccat ggagcagccg ttatttatag aactgcctgt tggaggtggg gagtctccc 1929

tccattcttg tccagaaaac tccttagctc tcgcagttag ccatgttctt agtctccagg 1989

gatggatggc cttgtatatg gacccctgag aatgagcaat tgagaaaaca aaacaaaagg 2049

aacaatccat gaacttagat tttattggtt tcaactcaaaa tgctgcagtc atttgacctg 2109

<210> 4

<211> 305

<212> PRT

<213> Homo sapiens

<400> 4

Met Arg Phe Met Gln Glu His Thr Cys Tyr Asp Ala Met Ala Thr Ser  
           1                                  5                                  10                                  15

Ser Lys Leu Val Ile Phe Asp Thr Met Leu Glu Ile Lys Lys Ala Phe  
                   20                                  25                                  30

Phe Ala Leu Val Ala Asn Gly Val Arg Ala Ala Pro Leu Trp Asp Ser  
                   35                                  40                                  45

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Lys Lys Gln Ser Phe Val Gly Met Leu Thr Ile Thr Asp Phe Ile Leu  
 50 55 60  
 Val Leu His Arg Tyr Tyr Arg Ser Pro Leu Val Gln Ile Tyr Glu Ile  
 65 70 75 80  
 Glu Gln His Lys Ile Glu Thr Trp Arg Glu Ile Tyr Leu Gln Gly Cys  
 85 90 95  
 Phe Lys Pro Leu Val Ser Ile Ser Pro Asn Asp Ser Leu Phe Glu Ala  
 100 105 110  
 Val Tyr Thr Leu Ile Lys Asn Arg Ile His Arg Leu Pro Val Leu Asp  
 115 120 125  
 Pro Val Ser Gly Asn Val Leu His Ile Leu Thr His Lys Arg Leu Leu  
 130 135 140  
 Lys Phe Leu His Ile Phe Gly Ser Leu Leu Pro Arg Pro Ser Phe Leu  
 145 150 155 160  
 Tyr Arg Thr Ile Gln Asp Leu Gly Ile Gly Thr Phe Arg Asp Leu Ala  
 165 170 175  
 Val Val Leu Glu Thr Ala Pro Ile Leu Thr Ala Leu Asp Ile Phe Val  
 180 185 190  
 Asp Arg Arg Val Ser Ala Leu Pro Val Val Asn Glu Cys Gly Gln Val  
 195 200 205  
 Val Gly Leu Tyr Ser Arg Phe Asp Val Ile His Leu Ala Ala Gln Gln  
 210 215 220  
 Thr Tyr Asn His Leu Asp Met Ser Val Gly Glu Ala Leu Arg Gln Arg  
 225 230 235 240  
 Thr Leu Cys Leu Glu Gly Val Leu Ser Cys Gln Pro His Glu Ser Leu  
 245 250 255  
 Gly Glu Val Ile Asp Arg Ile Ala Arg Glu Gln Val His Arg Leu Val  
 260 265 270  
 Leu Val Asp Glu Thr Gln His Leu Leu Gly Val Val Ser Leu Ser Asp  
 275 280 285  
 Ile Leu Gln Ala Leu Val Leu Ser Pro Ala Gly Ile Asp Ala Leu Gly  
 290 295 300  
 Ala  
 305

<210> 5  
 <211> 20  
 <212> DNA  
 <213> Sus scrofa

<400> 5  
 ggaatttcaa gtcagccaac

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<210> 6  
<211> 20  
<212> DNA  
<213> Sus scrofa

<400> 6  
cttcaaaaga ccgtgctact

20

<210> 7  
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<212> DNA  
<213> Sus scrofa

<400> 7  
ctgggaacct ctatatgctg

20

<210> 8  
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<400> 8  
tagggaaata caaatcacag

20

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<211> 20  
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<400> 9  
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20

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gtttctgcag ctttagcatc tattcc

26

<210> 11  
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<212> DNA  
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<400> 11  
gaagtatcct gggcttctga

20

<210> 12  
<211> 26  
<212> DNA  
<213> Sus scrofa

<400> 12

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gtttctccag gtttccagac atccac 26

<210> 13  
<211> 20  
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<213> Sus scrofa

<400> 13  
gcttctgtct gccctactt 20

<210> 14  
<211> 26  
<212> DNA  
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<400> 14  
gtttctaagt tctactgtaa gacacc 26

<210> 15  
<211> 20  
<212> DNA  
<213> Sus scrofa

<400> 15  
ccaagctgtg gtggctgaat 20

<210> 16  
<211> 20  
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<400> 16  
cagcacagca gtgccaccta 20

<210> 17  
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<212> DNA  
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<400> 17  
caaactcttc taggcgtgt 19

<210> 18  
<211> 26  
<212> DNA  
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<400> 18  
gtttctggaa cttccatatg ccatgg 26

<210> 19  
<211> 20  
<212> DNA  
<213> Sus scrofa

10/20

<400> 19  
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20

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<400> 20  
gtctcgctcc tgaaggaagt

20

<210> 21  
<211> 20  
<212> DNA  
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<400> 21  
agtcacgtgg ccattgctatc

20

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ctcaactgga ttgagtcagt

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ttggcgcaac tggtatttct

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aggcaaagga agagcacag

19

<210> 25  
<211> 18  
<212> DNA  
<213> Sus scrofa

<400> 25  
agccgtgggc atcgttgg

18

<210> 26  
<211> 21

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&lt;212&gt; DNA

&lt;213&gt; Sus scrofa

&lt;400&gt; 26

agaaggagac agacagggcga

21

&lt;210&gt; 27

&lt;211&gt; 1873

&lt;212&gt; ADN

&lt;213&gt; Sus scrofa

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1) .. (1395)

&lt;400&gt; 27

|   |     |
|---|-----|
| atg agc ttc cta gag caa gga gag agc cgt tca tgg cca tcc cga gct | 48  |
| Met Ser Phe Leu Glu Gln Gly Glu Ser Arg Ser Trp Pro Ser Arg Ala |     |
| 1 5 10 15   |     |
| gta acc acc agc tca gaa aga agc cat ggg gac cag ggg aac aag gcc | 96  |
| Val Thr Thr Ser Ser Glu Arg Ser His Gly Asp Gln Gly Asn Lys Ala |     |
| 20 25 30  |     |
| tct aga tgg aca agg cag gag gat gta gag gaa ggg ggg cct ccg ggc | 144 |
| Ser Arg Trp Thr Arg Gln Glu Asp Val Glu Glu Gly Gly Pro Pro Gly |     |
| 35 40 45  |     |
| ccg agg gaa ggt ccc cag tcc agg cca gtt gct gag tcc acc ggg cag | 192 |
| Pro Arg Glu Gly Pro Gln Ser Arg Pro Val Ala Glu Ser Thr Gly Gln |     |
| 50 55 60  |     |
| gag gcc aca ttc ccc aag gcc aca ccc ttg gcc caa gcc gct ccc ttg | 240 |
| Glu Ala Thr Phe Pro Lys Ala Thr Pro Leu Ala Gln Ala Ala Pro Leu |     |
| 65 70 75 80   |     |
| gcc gag gtg gac aac ccc cca aca gag cgg gac atc ctc ccc tct gac | 288 |
| Ala Glu Val Asp Asn Pro Pro Thr Glu Arg Asp Ile Leu Pro Ser Asp |     |
| 85 90 95  |     |
| tgt gca gcc tca gcc tcc gac tcc aac aca gac cat ctg gat ctg ggc | 336 |
| Cys Ala Ala Ser Ala Ser Asp Ser Asn Thr Asp His Leu Asp Leu Gly |     |
| 100 105 110   |     |
| ata gag ttc tca gcc tcg gcg gcg tcg ggg gat gag ctt ggg ctg gtg | 384 |
| Ile Glu Phe Ser Ala Ser Ala Ala Ser Gly Asp Glu Leu Gly Leu Val |     |
| 115 120 125   |     |
| gaa gag aag cca gcc ccg tgc cca tcc cca gag gtg ctg tta ccc agg | 432 |
| Glu Glu Lys Pro Ala Pro Cys Pro Ser Pro Glu Val Leu Leu Pro Arg |     |
| 130 135 140   |     |
| ctg ggc tgg gat gat gag ctg cag aag ccg ggg gcc cag gtc tac atg | 480 |
| Leu Gly Trp Asp Asp Glu Leu Gln Lys Pro Gly Ala Gln Val Tyr Met |     |
| 145 150 155 160   |     |
| cac ttc atg cag gag cac acc tgc tac gat gcc atg gcg acc agc tcc | 528 |
| His Phe Met Gln Glu His Thr Cys Tyr Asp Ala Met Ala Thr Ser Ser |     |
| 165 170 175   |     |

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|   |      |
|---|------|
| aaa ctg gtc atc ttc gac acc atg ctg gag atc aag aag gcc ttc ttt | 576  |
| Lys Leu Val Ile Phe Asp Thr Met Leu Glu Ile Lys Lys Ala Phe Phe |      |
| 180 185 190   |      |
| gcc ctg gtg gcc aac ggc gtc cga gcg gca cct ttg tgg gac agc aag | 624  |
| Ala Leu Val Ala Asn Gly Val Arg Ala Ala Pro Leu Trp Asp Ser Lys |      |
| 195 200 205   |      |
| aag cag agc ttc gtg ggg atg ctg acc atc aca gac ttc atc ttg gtg | 672  |
| Lys Gln Ser Phe Val Gly Met Leu Thr Ile Thr Asp Phe Ile Leu Val |      |
| 210 215 220   |      |
| ctg cac cgc tat tac agg tcc ccc ctg gtc cag atc tac gag att gaa | 720  |
| Leu His Arg Tyr Tyr Arg Ser Pro Leu Val Gln Ile Tyr Glu Ile Glu |      |
| 225 230 235 240   |      |
| gaa cat aag att gag acc tgg agg gag atc tac ctt caa ggc tgc ttc | 768  |
| Glu His Lys Ile Glu Thr Trp Arg Glu Ile Tyr Leu Gln Gly Cys Phe |      |
| 245 250 255   |      |
| aag cct ctg gtc tcc atc tct ccc aat gac agc ctg ttc gaa gct gtc | 816  |
| Lys Pro Leu Val Ser Ile Ser Pro Asn Asp Ser Leu Phe Glu Ala Val |      |
| 260 265 270   |      |
| tac gcc ctc atc aag aac cgg atc cac cgc ctg ccg gtc ctg gac cct | 864  |
| Tyr Ala Leu Ile Lys Asn Arg Ile His Arg Leu Pro Val Leu Asp Pro |      |
| 275 280 285   |      |
| gtc tcc ggg gct gtg ctc cac atc ctc aca cat aag cgg ctt ctc aag | 912  |
| Val Ser Gly Ala Val Leu His Ile Leu Thr His Lys Arg Leu Leu Lys |      |
| 290 295 300   |      |
| ttc ctg cac atc ttt ggc acc ctg ctg ccc cgg ccc tcc ttc ctc tac | 960  |
| Phe Leu His Ile Phe Gly Thr Leu Leu Pro Arg Pro Ser Phe Leu Tyr |      |
| 305 310 315 320   |      |
| cgc acc atc caa gat ttg ggc atc ggc aca ttc cga gac ttg gcc gtg | 1008 |
| Arg Thr Ile Gln Asp Leu Gly Ile Gly Thr Phe Arg Asp Leu Ala Val |      |
| 325 330 335   |      |
| gtg ctg gaa acg gcg ccc atc ctg acc gca ctg gac atc ttc gtg gac | 1056 |
| Val Leu Glu Thr Ala Pro Ile Leu Thr Ala Leu Asp Ile Phe Val Asp |      |
| 340 345 350   |      |
| cgg cgt gtg tct gcg ctg cct gtg gtc aac gaa act gga cag gta gtg | 1104 |
| Arg Arg Val Ser Ala Leu Pro Val Val Asn Glu Thr Gly Gln Val Val |      |
| 355 360 365   |      |
| ggc ctc tac tct cgc ttt gat gtg atc cac ctg gct gcc caa caa aca | 1152 |
| Gly Leu Tyr Ser Arg Phe Asp Val Ile His Leu Ala Ala Gln Gln Thr |      |
| 370 375 380   |      |
| tac aac cac ctg gac atg aat gtg gga gaa gcc ctg agg cag cgg aca | 1200 |
| Tyr Asn His Leu Asp Met Asn Val Gly Glu Ala Leu Arg Gln Arg Thr |      |
| 385 390 395 400   |      |
| ctg tgt ctg gaa ggc gtc ctt tcc tgc cag ccc cac gag acc ttg ggg | 1248 |
| Leu Cys Leu Glu Gly Val Leu Ser Cys Gln Pro His Glu Thr Leu Gly |      |
| 405 410 415   |      |

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gaa gtc att gac cgg att gtc cgg gaa cag gtg cac cgc ctg gtg ctc 1296  
 Glu Val Ile Asp Arg Ile Val Arg Glu Gln Val His Arg Leu Val Leu  
                   420                                  425                                  430

gtg gat gag acc cag cac ctt ctg ggc gtg gtg tcc ctc tct gac atc 1344  
 Val Asp Glu Thr Gln His Leu Leu Gly Val Val Ser Leu Ser Asp Ile  
                   435                                  440                                  445

ctt cag gct ctg gtg ctc agc cct gct gga att gat gcc ctc ggg gcc 1392  
 Leu Gln Ala Leu Val Leu Ser Pro Ala Gly Ile Asp Ala Leu Gly Ala  
                   450                                  455                                  460

tga gaaccttgga acctttgctc tcaggccacc tggcacacct ggaagccagt 1445  
 465

gaagggagcc gtggactcag ctctcacttc ccctcagccc cacttgctgg tctggctctt 1505  
 gttcaggtag gctccgcccc gggcccttgg cctcagcacc agccctcag tctccctggg 1565  
 caccagatc tcagactggg gcacctgaa gatgggagtg gccagctta tagctgagca 1625  
 gccttgtagaa atctaccagc atcaagactc actgtgggac cactgctttg tccattctc 1685  
 agctgaaatg atggagggcc tcataagagg ggtggacagg gcctggagta gaggccagat 1745  
 cagtgcgtg ccttcaggac ctccggggag ttagagctgc cctctctcag ttcagttccc 1805  
 ccctgctgag aatgtccctg gaaggaagcc agttaataaa ccttggttgg atggaatttg 1865  
 gagagtcg 1873

<210> 28  
 <211> 464  
 <212> PRT  
 <213> Sus scrofa

<400> 28  
 Met Ser Phe Leu Glu Gln Gly Glu Ser Arg Ser Trp Pro Ser Arg Ala  
   1                                  5                                  10                                  15  
 Val Thr Thr Ser Ser Glu Arg Ser His Gly Asp Gln Gly Asn Lys Ala  
                   20                                  25                                  30  
 Ser Arg Trp Thr Arg Gln Glu Asp Val Glu Glu Gly Gly Pro Pro Gly  
                   35                                  40                                  45  
 Pro Arg Glu Gly Pro Gln Ser Arg Pro Val Ala Glu Ser Thr Gly Gln  
                   50                                  55                                  60  
 Glu Ala Thr Phe Pro Lys Ala Thr Pro Leu Ala Gln Ala Ala Pro Leu  
                   65                                  70                                  75                                  80  
 Ala Glu Val Asp Asn Pro Pro Thr Glu Arg Asp Ile Leu Pro Ser Asp  
                   85                                  90                                  95  
 Cys Ala Ala Ser Ala Ser Asp Ser Asn Thr Asp His Leu Asp Leu Gly  
                   100                                  105                                  110  
 Ile Glu Phe Ser Ala Ser Ala Ala Ser Gly Asp Glu Leu Gly Leu Val  
                   115                                  120                                  125  
 Glu Glu Lys Pro Ala Pro Cys Pro Ser Pro Glu Val Leu Leu Pro Arg  
                   130                                  135                                  140  
 Leu Gly Trp Asp Asp Glu Leu Gln Lys Pro Gly Ala Gln Val Tyr Met  
                   145                                  150                                  155                                  160  
 His Phe Met Gln Glu His Thr Cys Tyr Asp Ala Met Ala Thr Ser Ser  
                   165                                  170                                  175

SUBSTITUTE SHEET (RULE 26)



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Lys Leu Val Ile Phe Asp Thr Met Leu Glu Ile Lys Lys Ala Phe Phe  
 180 185 190  
 Ala Leu Val Ala Asn Gly Val Arg Ala Ala Pro Leu Trp Asp Ser Lys  
 195 200 205  
 Lys Gln Ser Phe Val Gly Met Leu Thr Ile Thr Asp Phe Ile Leu Val  
 210 215 220  
 Leu His Arg Tyr Tyr Arg Ser Pro Leu Val Gln Ile Tyr Glu Ile Glu  
 225 230 235 240  
 Glu His Lys Ile Glu Thr Trp Arg Glu Ile Tyr Leu Gln Gly Cys Phe  
 245 250 255  
 Lys Pro Leu Val Ser Ile Ser Pro Asn Asp Ser Leu Phe Glu Ala Val  
 260 265 270  
 Tyr Ala Leu Ile Lys Asn Arg Ile His Arg Leu Pro Val Leu Asp Pro  
 275 280 285  
 Val Ser Gly Ala Val Leu His Ile Leu Thr His Lys Arg Leu Leu Lys  
 290 295 300  
 Phe Leu His Ile Phe Gly Thr Leu Leu Pro Arg Pro Ser Phe Leu Tyr  
 305 310 315 320  
 Arg Thr Ile Gln Asp Leu Gly Ile Gly Thr Phe Arg Asp Leu Ala Val  
 325 330 335  
 Val Leu Glu Thr Ala Pro Ile Leu Thr Ala Leu Asp Ile Phe Val Asp  
 340 345 350  
 Arg Arg Val Ser Ala Leu Pro Val Val Asn Glu Thr Gly Gln Val Val  
 355 360 365  
 Gly Leu Tyr Ser Arg Phe Asp Val Ile His Leu Ala Ala Gln Gln Thr  
 370 375 380  
 Tyr Asn His Leu Asp Met Asn Val Gly Glu Ala Leu Arg Gln Arg Thr  
 385 390 395 400  
 Leu Cys Leu Glu Gly Val Leu Ser Cys Gln Pro His Glu Thr Leu Gly  
 405 410 415  
 Glu Val Ile Asp Arg Ile Val Arg Glu Gln Val His Arg Leu Val Leu  
 420 425 430  
 Val Asp Glu Thr Gln His Leu Leu Gly Val Val Ser Leu Ser Asp Ile  
 435 440 445  
 Leu Gln Ala Leu Val Leu Ser Pro Ala Gly Ile Asp Ala Leu Gly Ala  
 450 455 460

&lt;210&gt; 29

&lt;211&gt; 2115

&lt;212&gt; ADN

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(1395)

&lt;400&gt; 29

atg agc ttc cta gag caa gaa aac agc agc tca tgg cca tca cca gct 48  
 Met Ser Phe Leu Glu Gln Glu Asn Ser Ser Ser Trp Pro Ser Pro Ala  
 1 5 10 15

gtg acc agc agc tca gaa aga atc cgt ggg aaa cgg agg gcc aaa gcc 96  
 Val Thr Ser Ser Ser Glu Arg Ile Arg Gly Lys Arg Arg Ala Lys Ala  
 20 25 30

ttg aga tgg aca agg cag aag tcg gtg gag gaa ggg gag cca cca ggt 144  
 Leu Arg Trp Thr Arg Gln Lys Ser Val Glu Glu Gly Glu Pro Pro Gly  
 35 40 45

15/20

|   |     |
|---|-----|
| cag ggg gaa ggt ccc cgg tcc agg cca act gct gag tcc acc ggg ctg | 192 |
| Gln Gly Glu Gly Pro Arg Ser Arg Pro Thr Ala Glu Ser Thr Gly Leu |     |
| 50 55 60  |     |
| gag gcc aca ttc ccc aag acc aca ccc ttg gct caa gct gat cct gcc | 240 |
| Glu Ala Thr Phe Pro Lys Thr Thr Pro Leu Ala Gln Ala Asp Pro Ala |     |
| 65 70 75 80   |     |
| ggg gtg ggc act cca cca aca ggg tgg gac tgc ctc ccc tct gac tgt | 288 |
| Gly Val Gly Thr Pro Pro Thr Gly Trp Asp Cys Leu Pro Ser Asp Cys |     |
| 85 90 95  |     |
| aca gcc tca gct gca ggc tcc agc aca gat gat gtg gag ctg gcc acg | 336 |
| Thr Ala Ser Ala Ala Gly Ser Ser Thr Asp Asp Val Glu Leu Ala Thr |     |
| 100 105 110   |     |
| gag ttc cca gcc aca gag gcc tgg gag tgt gag cta gaa ggc ctg ctg | 384 |
| Glu Phe Pro Ala Thr Glu Ala Trp Glu Cys Glu Leu Glu Gly Leu Leu |     |
| 115 120 125   |     |
| gaa gag agg cct gcc ctg tgc ctg tcc ccg cag gcc cca ttt ccc aag | 432 |
| Glu Glu Arg Pro Ala Leu Cys Leu Ser Pro Gln Ala Pro Phe Pro Lys |     |
| 130 135 140   |     |
| ctg ggc tgg gat gac gaa ctg cgg aaa ccc ggc gcc cag atc tac atg | 480 |
| Leu Gly Trp Asp Asp Glu Leu Arg Lys Pro Gly Ala Gln Ile Tyr Met |     |
| 145 150 155 160   |     |
| cgc ttc atg cag gag cac acc tgc tac gat gcc atg gca act agc tcc | 528 |
| Arg Phe Met Gln Glu His Thr Cys Tyr Asp Ala Met Ala Thr Ser Ser |     |
| 165 170 175   |     |
| aag cta gtc atc ttc gac acc atg ctg gag atc aag aag gcc ttc ttt | 576 |
| Lys Leu Val Ile Phe Asp Thr Met Leu Glu Ile Lys Lys Ala Phe Phe |     |
| 180 185 190   |     |
| gct ctg gtg gcc aac ggt gtg cgg gca gcc cct cta tgg gac agc aag | 624 |
| Ala Leu Val Ala Asn Gly Val Arg Ala Ala Pro Leu Trp Asp Ser Lys |     |
| 195 200 205   |     |
| aag cag agc ttt gtg ggg atg ctg acc atc act gac ttc atc ctg gtg | 672 |
| Lys Gln Ser Phe Val Gly Met Leu Thr Ile Thr Asp Phe Ile Leu Val |     |
| 210 215 220   |     |
| ctg cat cgc tac tac agg tcc ccc ctg gtc cag atc tat gag att gaa | 720 |
| Leu His Arg Tyr Tyr Arg Ser Pro Leu Val Gln Ile Tyr Glu Ile Glu |     |
| 225 230 235 240   |     |
| caa cat aag att gag acc tgg agg gag atc tac ctg caa ggc tgc ttc | 768 |
| Gln His Lys Ile Glu Thr Trp Arg Glu Ile Tyr Leu Gln Gly Cys Phe |     |
| 245 250 255   |     |
| aag cct ctg gtc tcc atc tct cct aat gat agc ctg ttt gaa gct gtc | 816 |
| Lys Pro Leu Val Ser Ile Ser Pro Asn Asp Ser Leu Phe Glu Ala Val |     |
| 260 265 270   |     |
| tac acc ctc atc aag aac cgg atc cat cgc ctg cct gtt ctt gac ccg | 864 |
| Tyr Thr Leu Ile Lys Asn Arg Ile His Arg Leu Pro Val Leu Asp Pro |     |
| 275 280 285   |     |

16/20

|   |      |
|---|------|
| gtg tca ggc aac gta ctc cac atc ctc aca cac aaa cgc ctg ctc aag   | 912  |
| Val Ser Gly Asn Val Leu His Ile Leu Thr His Lys Arg Leu Leu Lys   |      |
| 290 295 300   |      |
| ttc ctg cac atc ttt ggt tcc ctg ctg ccc cgg ccc tcc ttc ctc tac   | 960  |
| Phe Leu His Ile Phe Gly Ser Leu Leu Pro Arg Pro Ser Phe Leu Tyr   |      |
| 305 310 315 320   |      |
| cgc act atc caa gat ttg ggc atc ggc aca ttc cga gac ttg gct gtg   | 1008 |
| Arg Thr Ile Gln Asp Leu Gly Ile Gly Thr Phe Arg Asp Leu Ala Val   |      |
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| gtg ctg gag aca gca ccc atc ctg act gca ctg gac atc ttt gtg gac   | 1056 |
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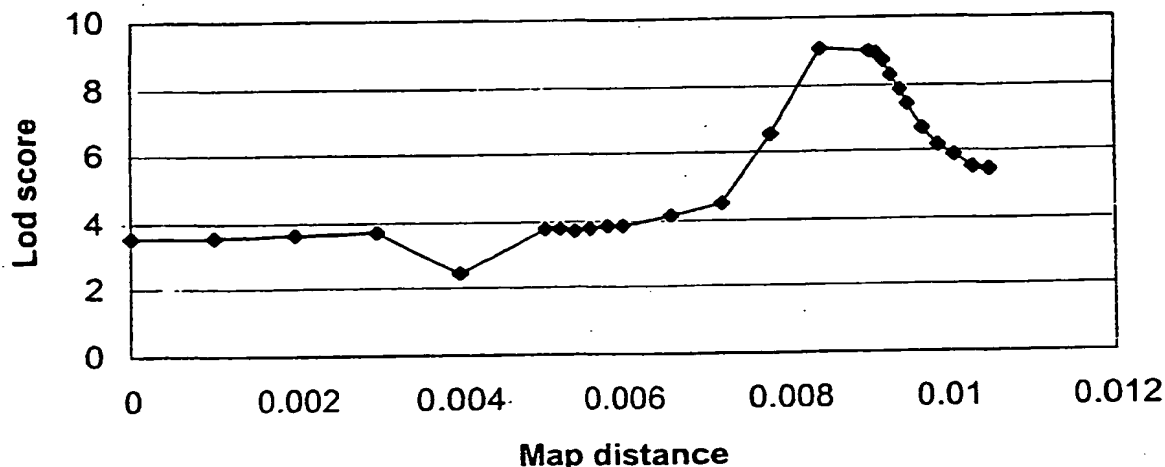
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CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— With international search report.

[Continued on next page]

(54) Title: VARIANTS OF THE GAMMA CHAIN OF AMPK, DNA SEQUENCES ENCODING THE SAME, AND USES THEREOF



(57) Abstract: The invention concerns variants of the gamma chain of vertebrate AMP-activated kinase (AMPK), as well as nucleic acid sequences encoding said variants and use thereof for the diagnosis or treatment of dysfunction of energy metabolisms.

WO 01/20003 A3



— Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.

For letter codes and other abbreviations, refer to the "Guidance on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

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## A. CLASSIFICATION OF SUBJECT MATTER

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G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C12Q A01K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

STRAND, EMBL

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. |
|------------|---|-----------------------|
| X          | HILLIER ET AL.: "WashU-NCI human EST project"<br>EMBL DATABASE ACC NO: AA178898,<br>1 January 1997 (1997-01-01), XP002130593<br>cited in the application<br>abstract  | 1-5,<br>11-17         |
| X          | ROBIC ET AL.: "A radiation hybrid map of the RN region in pigs demonstrates conserved gene order compared with the human and mouse genomes"<br>MAMMALIAN GENOME,<br>vol. 10, no. 6, June 1999 (1999-06), pages 565-568, XP000876695<br>cited in the application<br>page 565 | 29-33                 |
| A          | page 567; figure 1; table 1<br>---<br>-/--  | 1-28,<br>32-37        |

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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- \*O\* document referring to an oral disclosure, use, exhibition or other means
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- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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- \*&\* document member of the same patent family

Date of the actual completion of the international search

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Fax: (+31-70) 340-3016

Authorized officer

van Klompenburg, W

## INTERNATIONAL SEARCH REPORT

International Application No

PC./EP 00/09896

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| Category | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. |
|----------|---|-----------------------|
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| A        | claims 1-24; figure 7   | 1-12,<br>16-37        |
| X        | WATERSTON: "Homo sapiens chromosome<br>unknown clone NH0459I19"<br>EMBL DATABASE ACC NO: AC009974,<br>9 September 1999 (1999-09-09),<br>XP002130594<br>abstract   | 11-16                 |
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| A        | MILAN ET AL.: "Accurate mapping of the<br>"acid meat" RN gene on genetic and<br>physical maps of pig chromosome 15"<br>MAMMALIAN GENOME,<br>vol. 7, no. 1, January 1996 (1996-01),<br>pages 47-51, XP000876743<br>cited in the application<br>page 50, column 2 -page 51, column 1;<br>figure 1 | 1-35                  |
|          | ---   |                       |
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## INTERNATIONAL SEARCH REPORT

International Application No

PC../EP 00/09896

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No.   |
|------------|---|-------------------------|
| P,X        | <p>CHEUNG ET AL.: "Characterization of<br/>AMP-activated protein kinase gamma-subunit<br/>isoforms and their role in AMP binding"<br/>BIOCHEMICAL JOURNAL,<br/>vol. 346; no. 3,<br/>15 March 2000 (2000-03-15), pages 659-669,<br/>XP002162237<br/>figures 2,3,5; tables 1,2<br/>-&amp; DATABASE EMBL 'Online!<br/>EBI;<br/>ACC. NO.: AJ249977,<br/>7 January 2000 (2000-01-07)<br/>CARLING : "Homo sapiens mRNA for<br/>AMP-activated protein kinase gamma 3<br/>subunit (AMPK gamma 3 gene)"<br/>XP002162239<br/>abstract</p> <p>----</p> | 1-4,<br>11-17,<br>20,37 |
| P,X        | <p>MILAN ET AL.: "A mutation in PRKAG3<br/>associated with excess glycogen content in<br/>pig skeletal muscle"<br/>SCIENCE,<br/>vol. 288, 19 May 2000 (2000-05-19), pages<br/>1248-1251, XP002162238<br/>figures 1,2; tables 1-3</p> <p>-----</p>   | 1-33                    |

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Information on patent family members

International Application No

PC../EP 00/09896

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s) | Publication<br>date |
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